1,4-Dihydropyridines: Reactivity of Nitrosoaryl and Nitroaryl Derivatives with Alkylperoxyl Radicals and ABTS Radical Cation

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In the present paper, a direct quenching of radical species by a number of synthesized nitrosoaryl 1,4-dihydropyridines and their parent nitroaryl 1,4-dihydropyridines was determined in aqueous media at pH 7.4. These two series of compounds were compared with the C-4 unsubstituted 1,4-dihydropyridines derivatives and the corresponding C-4 aryl substituted 1,4-dihydropyridines derivatives. Kinetic rate constants were assessed by UV-Vis spectroscopy. Nitrosoaryl derivatives were more reactive than the parent nitroaryl 1,4-dihydropyridines.

Our results strongly support the assumption that the reactivity between the synthesized 1,4-dihydropyridines derivatives with alkylperoxyl radicals involves electron transfer reactions, which is documented by the presence of pyridine as final product of reaction and the complete oxidation of the nitroso group to give rise the nitro group in the case of the nitrosoaryl 1,4-dihydropyridines derivatives.

Keywords: Aryl 1,4-dihydropyridines; UV-Vis spectroscopy; Nitroaryl 1,4-dihydropyridines; Britton–Robinson buffer

INTRODUCTION

Calcium channel antagonists are a heterogenous group of compounds active in inhibiting the inward flux of calcium by interacting with potential-operated channels.[1] A particular class of this type of compounds is constituted by N-unsubstituted 1,4-dihydropyridines, which interact with the a-1 subunit of the L-type Ca^{+2} channels and are among the most active compounds in the treatment of hypertension.[2]

Concerning the mechanism of action, it has been concluded that 1,4-dihydropyridines cause vaso-relaxation by two mechanisms: (a) directly, by blocking voltage-operated L-type calcium channel in smooth muscle cells and (b) indirectly, by increasing NO release from intact endothelium.[3,4]

Hantzsch 1,4-dihydropyridines continue to attract much attention not only for their pharmacological effects but also as a tool for the investigation of the calcium channel[5] and its use as NAD(P)H models to probe the mechanism of hydrogen transfer.[6]

It is well established that some 1,4-dihydropyridines behave as antioxidants.[7–10] Concerning this action, in a recent paper,[11] we reported the direct interaction between some synthesized 1,4-DHP derivatives with alkylperoxyl radicals and ABTS radical cation. In addition, the structure–reactivity relationship between these derivatives and the free radicals has also been depicted.[11] Furthermore, the photodegradation of nitroaryl 1,4-dihydropyrdines which gives rise to nitroso derivatives has also been well-documented.[12,13] Thus, nifedipine, unstable under daylight conditions, is converted into a nitrosopyridine derivative,[14,15] which reacts with unsaturated lipid, thus forming stable nitroxide radicals covalently attached to membrane lipids and preventing its lipoperoxidation.[14] Also, we have reported that some commercial 1,4-dihydropyridines and related nitrosoaryl derivatives have the ability to prevent lipid peroxidation in rat brain

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slices in a nonenzymatic active oxygen-generating system.[16] Therefore, investigating the reactivity of a number of new synthesized nitrosoaryl 1,4-dihydropyridines and their corresponding parent nitroaryl 1,4-dihydropyridine derivatives with free radicals constitutes an appealing challenge.

In the present study, we examined the ability of 10 synthesized nitrosoaryl derivatives and their parent nitroaryl 1,4-dihydropyridines to react with alkylperoxyl radicals and ABTS radical cation at pH 7.4. The results obtained from the tested compounds were compared with Trolox and nisoldipine. UV-Vis spectroscopy, GC/MS, HPLC and electrochemical techniques were used to obtain experimental data to follow the reactivity of the compounds and to support some details on the reaction mechanism.

MATERIALS AND METHODS

Chemicals

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

Compounds

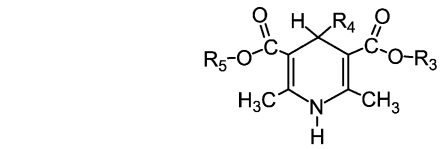
Synthesis 1,4-dihydropyridine derivatives (Fig. 1) was based on classical Hantzsch synthesis of 1,4-dihydropyridines.[17,18]

General Procedure

A mixture of 0.079 mol of alkyl acetoacetate (methyl, ethyl, isopropyl) and 10 ml of concentrated ammonium hydroxide in 20 ml of ethyl alcohol was heated under reflux for 2.5 h. The resulting clear solution was added to a mixture of 0.079 mol of alkyl acetoacetate, 0.165 mol of aldehyde (3 or 4-nitrobenzaldehyde), 25 ml of concentrated ammonium hydroxide and 20 ml of ethyl alcohol and refluxed for 15 h. The crude solid product was filtered and recrystallized in ethyl alcohol. The yields were in the 80–90% range depending upon the derivative.

Preparation of Nitrosophenyl Derivatives

The corresponding 4-(3 or 4-nitrophenyl)-2,6-dimethyl-3,5-dialkoxycarbonyl-1,4-dihydropyridine derivatives (2.1×10^{-2} mol) were dissolved in absolute EtOH (50 ml) in a round bottom flask. Calcium chloride (3 g) previously dissolved in the minimum amount of distilled water was added and then powdered Zn (2 g) was also added in small amounts to the stirred solution. This took about 5 min; the solution turned orange, corresponding to a solution of the hydroxylamine compound. Once all the Zn was added, the system was gently refluxed



Compound	R_3	R_4	R_5
I	$-CH(CH_3)_2$	NO	$-CH(CH_3)_2$
II	$-CH(CH_3)_2$	NO	$-CH(CH_3)_2$
III	$-CH_2CH_3$	NO	$-CH_2CH_3$
IV	$-CH_3$	NO	$-CH_3$
V	$-CH_2CH_3$	NO	$-CH_2CH_3$
VI	$-CH_3$	O_2N	$-CH_3$
VII	$-CH_3$	NO_2	$-CH_3$
VIII	$-CH_3$	NO_2	$-CH_3$
IX	$-CH_2CH_3$	$-H$	$-CH_2CH_3$
X	$-CH_3$		$-CH_3$
XI (Nisoldipine)	$-CH_2-CH(CH_3)_2$	O_2N	$-CH_3$

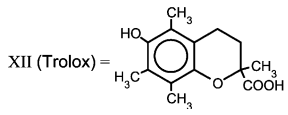


FIGURE 1 Chemical structures of 1,4-DHP derivatives.

for 15 min with vigorous stirring. The warm solution was filtered to take off the ZnO formed. The filtered and cold solution was added as fast as possible to an ice cold water solution containing previously prepared $FeCl_3$ (7 g). The resulting mixture was kept at 0°C for 10 min and a green precipitate formed corresponding to the crude nitroso compound. The precipitate was filtered and dried in a vacuum desiccators. Toluene (10–20 ml) was added to the dry precipitate and stirred to form an homogeneous paste. The paste was poured into a 20 cm silica gel 60 chromatographic column (mobile phase: toluene: ethyl acetate 9:1). The eluted greenish portion was collected and concentrated until the appearance of green crystals. The solution was then cooled and remained at rest for 24 h protected from sunlight and in an argon atmosphere to prevent decomposition of

the compound. The crystals were filtered, dried and stored in an argon atmosphere in a vessel protected from the sunlight.

All the synthesized compounds were characterized by ^{1}H-NMR, ^{13}C-NMR spectroscopy using 300 MHz spectrometer (Bruker, WM 300), infrared spectroscopy (FT-IR Paragon Spectrometer, 100PC) and Elemental analysis (Fison).

Compound I: 4-(3-Nitrosophenyl)-2,6-dimethyl-3,5-diisopropyloxycarbonyl-1,4-dihydropyridine

mp: 130.7°C. IR (KBr): ν_{max} 3347.9, 2979.4, 1699.3, 1648.2, 1489.0, 1369.8, 1295.7, 1216.3, 1104.6, 1016.1, 750.0 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.0 (d, 6H, $J = 6,24$ Hz, >CH-$(CH_3)_2$), 1.18 (d, 6H, $J = 6,4$ Hz, >CH-$(CH_3)_2$), 2.27 (s, 6H, -R-CH_3), 4.86 (m, 2H, $J =$ 6.24 Hz, -CH$(CH_3)_2$), 5.02 (s, 1H, >CH-), 5.82 (s, 1H, >NH), 7.50, (m, 4H, Ar-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 18.551; 20.766; 21.085; 39.038; 39.155; 66.229; 66.306; 102.600; 102.804; 118.400; 119.665; 120.208; 122.319; 127.436; 133.663; 134.715; 143.474; 146.997; 148.823; 165.757; 167.849 ppm. Calc. $C_{21}H_{26}O_5N_2$: C = 65.27; H = 6.78; N = 6.961. Found: C = 63.63; H = 7.03; N = 6.75.

Compound II: 4-(4-Nitrosophenyl)-2,6-dimethyl-3,5-diisopropiloxycarbonyl-1,4-dihydropyridine

mp: 101.2°C. IR (KBr): ν_{max} 3309.4, 2981.8, 1699.7, 1649.1, 1488.8, 1344.9, 1300.8, 1218.1, 1103.7, 1016.3, 833.1 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11 (d, 6H, $J = 6.24$ Hz, -CH-$(CH_3)_2$), 1.26 (d, 6H, $J = 6.24$ Hz, -CH-$(CH_3)_2$), 2.35 (s, 6H, -R-CH_3), 4.96 (m, 2H, $J =$ 6.24 Hz, -CH$(CH_3)_2$), 5.07 (s, 1H, >CH-), 5.85 (s, 1H, -R-NH), 7.48 (d, 2H, $J = 8.76$ Hz, Ar-H); 8.11 (d, 2H, $J = 8.5$ Hz, Ar-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 14.274; 19.687; 21.866; 21.116; 40.287; 40.603; 67.402; 103.270; 103.417; 121.063; 123.187; 128.236; 129.098; 144.449; 146.138; 155.345; (2 × 156.057); 165.658; 166.636; 166.695 ppm. Calc. $C_{21}H_{26}O_5N_2$: C = 65.27; H = 6.78; N = 6.961. Found: C = 66.866; H = 6.959; N = 6.986.

Compound III: 4-(4-Nitrosophenyl)-2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine

mp: 87.2–88.4°C. IR (KBr): ν_{max} 3342.5, 2997.8, 1671.5, 1486.7, 1369.2, 1303.2, 1217.3, 1120.1, 1020.3, 810.4 cm^{-1}. ^{1}H-RMN: δ1.21(t, 6H, $J = 7.1$ Hz, CH_2CH_3), 2.36(s, 6H, R-CH_3), 4.1(q, 4H, $J = 7.1$ Hz, -CH_2-CH_3), 5.1(s, 1H, R-CH), 5.7(s, 1H, >NH), 7.5(d, 2H, $J = 8.26$ Hz, Ar-H), 7.7(d, 2H, $J = 8.6$ Hz, Ar-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): (2 × 14.242); (2 × 19.657); (2 × 21.436); 40.437; 59.977; 103.095; 121.108; 125.286; 128.214; 128.859; 129.026; 144.588; (2 × 155.740); 165.632; 167.098 ppm. Calc. $C_{19}H_{22}O_5N_2$: C = 63.67; H = 6.286; N = 7.616. Found: C = 66.195; H = 6.549; N = 6.959.

Compound IV: 4-(4-Nitrosophenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine

mp: 147.0°C. IR (KBr): ν_{max} 3335, 2951, 1704, 1650, 1488, 1435, 1020 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.37 (s, 6H, R-CH_3), 3.65 (s, 6H, -O-CH_3), 5.10 (s, 1H, >CH-), 5.86 (s, 1H, >NH), 7.52 (d, 2H, $J = 8.56$ Hz, Ar-H), 7.79 (d, 2H, $J = 8.56$ Hz, Ar-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): (2 × 18.331); 19.565; 40.011; 51.068; (2 × 58.378); 102.729; (2 × 121.183); (2 × 128.450); 144.869; (2 × 155.315); 165.469; 167.445 ppm. Calc. $C_{17}H_{18}O_5N_2$: C = 61.81; H = 5.49; N = 8.48. Found: C = 62.22; H = 5.63; N = 8.61.

Compound V: 4-(3-Nitrosophenyl)-2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine

mp: 118.2–119.1°C. IR (KBr): ν_{max} 3334.5, 29981.7, 1699.8, 1651.3, 1488.8, 1371, 1300.2, 1213.3, 1102.1, 1020.2 cm^{-1}. ^{1}H-RMN (300 MHz, $CDCl_3$): δ1.2(t, 6H, $J = 7.2$ Hz, -CH_2CH_3), 2.36(s, 6H, R-CH_3), 4.1(m, 4H, -CH_2CH_3), 5.1(s, 1H, >CH-), 5.7(s, 1H, >NH), 7.7(m, 4H, Ar-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): (2 × 13.2302); (2 × 18.5819); 38.852; 58.9277, 102.533; 118.131; 119.819; 127.550; 128.035; 134.542; 143.681; (2 × 148.692); 165.457; 165.796; 166.248; 167.671 ppm. Calc. $C_{19}H_{22}O_5N_2$: C = 63.67; H = 6.286; N = 7.616. Found: C = 63.52; H = 6.48; N = 7.46.

Compound VI: 4-(2-Nitrophenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine

mp. 169.0°C. IR (KBr): ν_{max} 3331, 2920, 1681, 1530, 1495, 1433, 1350, 1022 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 6H, -CH_3), 3.60 (s, 6H, -O-CH_3), 5.73 (s, 1H, Ar-CH<), 5.91 (s, 1H, -NH-), 7.28 (m, 1H, Ar-H), 7.54 (m, 2H, Ar-H), 7.70 (d, 1H, $J = 8.1$ Hz, Ar-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): (19.435 × 2); (34.390 × 2); (50.976 × 2); (103.527 × 2); 123.829; 126.979; 130.965; 132.690; 142.030; 144.887; 147.731; (167.489 × 2) ppm. Calc. $C_{17}H_{18}O_6N_2$: C = 58.96; H = 5.24;N = 8.09. Found: C = 58.83; H = 5.18; N = 8.22.

Compound VII: 4-(3-Nitrophenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine

mp: 208.0°C. IR (KBr): ν_{max} 3350, 2950, 1705, 1646, 1529, 1486, 1431, 1347, 1127, 1100 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 6H, -CH_3), 3.65 (s, 6H, -O-CH_3), 5.11 (s, 1H, Ar-CH<), 5.95 (s, 1H, -NH-), 7.40 (m, 1H, Ar-H), 7.65 (d, 1H, $J = 8.1$ Hz, Ar-H), 8.02 (d, 1H, $J = 8.1$ Hz, Ar-H), 8.09 (s, 1H, Ar-H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): (19.508 × 2); (39.557 × 2); 51.060; 103.026; 121.327; 122.640;

128.646; 129.026; 132.954; 134.115; 144.938; 148.300; 149.488; (167.455 × 2) ppm. Calc. $C_{17}H_{18}O_6N_2$: C = 58.96; H = 5.24; N = 8.09. Found: C = 58.90; H = 5.21; N = 8.18.

Compound VIII: 4-(4-Nitrophenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine

mp: 197.0°C. IR (KBr): ν_{max} 3333, 2920, 1682, 1530, 1495, 1434, 1021 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.38 (s, 6H, -CH_3), 3.66 (s, 6H, -O-CH_3), 5.12 (s, 1H, Ar-CH <), 5.85 (s, 1H, -NH-), 7.46 (d, 2H, J = 8.06Hz, Ar-H), 8.12 (d, 2H, J = 8.06 Hz, Ar-H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): (19.588 × 2); (39.725 × 2); 51.100; 102.868; 123.377; 123.702; 123.781; 128.520; 129.390; 129.514; 144.859; 146.272; 154.672; (167.386 × 2) ppm. Calc. $C_{17}H_{18}O_6N_2$: C = 58.96; H = 5.24; N = 8.09. Found: C = 58.89; H = 5.17; N = 8.01.

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

UV-Visible Spectroscopic Experiments

The degree of progress in 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 was followed at λ = 734 nm.

The acquisition and treatment of data were carried out with a Vision 2.11 software.

Reactivity towards Alkylperoxyl Radicals

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

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 either 1,4-dihydropyridine or Trolox at 37°C for 120 min with constant bubbling of oxygen. The rate of alkylperoxyl radical 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 should be approximately constant, i.e. 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.[19,20]

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 synthesized 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 λ = 318–360 and 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, i.e. 4.92 × 10^3 M^{-1} s^{-1} taken from Ref. [21]

Control solutions (in the absence of ABAP-derived radicals) revealed no change either in their original UV-Vis absorption bands or during GC/MS mass fragmentation.

Besides, a possible photodecomposition of 1,4-dihydropyridines was evaluated, but this was negligible because of the time-scale of the experiments.

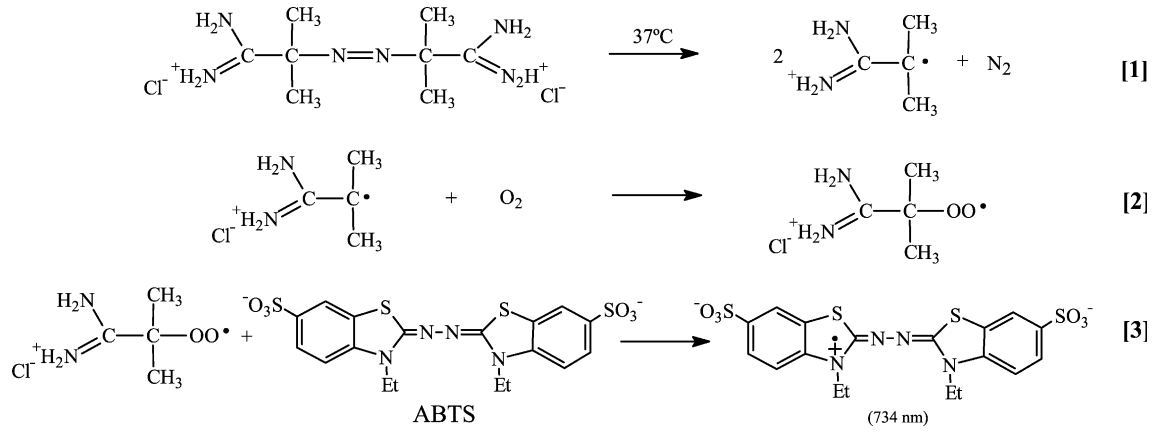


FIGURE 2 Chemical generation of free radicals: Equation [2]: alkylperoxyl radicals. Equation [3] $ABTS^+$ radical cation.

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. 2), a time period that generally proves adequate to produce an increase in the absorbance at 734 nm near 0.24 AU (in the absence of ABAP, incubated ABTS generated no color). By using an extinction coefficient of $1.6 \times 10^4\,mol^{-1}\,l\,cm^{-1}$ for ABTS radical cation at 734 nm,[22] we calculated the ABTS radical cation concentration formed in our experiments. The calculated value was 15 μM, indicating that a 20% ABTS was oxidized.

Afterwards, the resulting colored solution was rapidly cooled down on ice, nitrogen-bubbled for 5 min, and kept at 4°C until use. When aliquots (2 ml) of the colored 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.

The reactivity towards alkylperoxyl radicals and ABTS radical cation was comparatively expressed either with Trolox or nisoldipine using the following ratio.

Relative Reactivity

kinetic rate constant 1,4-DHP tested/kinetic rate constant Trolox or Nisoldipine.

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.

Controlled Potential Electrolysis (CPE)

CPE were carried out on a reticulate glassy carbon electrode in 0.04 M Britton–Robinson buffer/DMF 70/30 pH 7.4 between +0.95 and +1.0 V. 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.

GC/MS

A Gas Chromatograph/Mass Selective Hewlett Packard 5890/5972 Detector (Palo Alto, California, USA) and a Hewlett Packard 7673 Autosampler were used for the measurements. A Hewlett Packard Pentium II Data System-Laser Jet 4000 printer, controlled instrumentation and data handling were 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 (held for 5 min) at $15°C\,min^{-1}$; run time was 16.67 min. Helium was used as the carrier gas with an inlet pressure of 35 kPa. The mass range monitored was 45–550 amu with a scan rate of 1 scan/s and the ionization energy was set at 70 eV.

Peak identification relied upon full spectra comparison of the test samples with pure synthesized drugs. There must be a complete agreement with both acceptable chromatography and mass spectrometry. After the reaction, the final solutions were diluted and injected without any previous treatment. Consequently, no extraction or filtration procedures were necessary.

HPLC Analysis on the Oxidation of 1,4-DHP after the Reaction with Free Radicals. Equipment and Operation Conditions

HPLC measurements were carried out by using a Waters assembly equipped with a model 600 controller pump and a model 996 photodiode array (PDA) detector. The acquisition and treatment of data were performed by means of the Millennium version 2.1 software. The chromatographic column was a μBondapak/μPorasil C-18 column (3.9 × 150 mm) while the precolumn was a C-18 μBondapak precolumn (30 × 4.6 mm). The injector was a 20 μl Rheodyne valve.

An aliquot of 20 μl of each 1,4-dihydropyridine solutions which reacted with alkylperoxyl radicals

for 30–60 min was taken and injected into the chromatographic system.

Mobile phase: (a) For nitrosoaryl 1,4-DHP derivatives and compound IX, a mixture of Methanol/0.05 M phosphate buffer (60/40) at pH 4.3 was used as the mobile phase. (b) For nitroaryl 1,4-DHP derivatives and compound X, a mixture of Methanol/0.05 M phosphate buffer (50/50) at pH 4.1 was used as the mobile phase.

The PDA detector was operated at 250 nm for quantification. The flow of the mobile phase was maintained at 1.2 ml min^{-1} and a helium bubbling of 30 ml min^{-1} was applied to remove dissolved gases at 35°C temperature.

RESULTS AND DISCUSSION

Reactivity towards Alkylperoxyl Radicals

UV-Vis spectroscopy, GC/MS and HPLC technique were used to follow the reactivity of 1,4-DHP derivatives.

For the spectroscopic studies, the original UV absorption bands between $\lambda = 318$ and 360 nm were followed to evaluate reactivity. The results revealed that after the addition of 1,4-DHP derivatives to an aqueous mixture containing alkylperoxyl radicals, the absorption bands decreased along time (Fig. 3), parallel with the appearance of a new band between 270 and 280 nm (Fig. 3, Insert). This latter signal could correspond to the oxidized derivative, i.e. the pyridine derivative, which agrees with previous observations.[23]

The rate of reaction exhibited a linear dependence with concentration in the range of 20–120 μM concentration of 1,4-DHP.

To compare the reactivity, kinetic rate constants of tested 1,4-DHP derivatives and kinetic rate constants of the corresponding reference compounds (Trolox or Nisoldipine) were used (Table I). Clearly, all tested 1,4-DHP derivatives reacted with alkylperoxyl radicals at varied rates. Nitrosoaryl 1,4-DHP (Compounds I–V) were 6 times more reactive than the nitroaryl compounds (Compounds VI–VIII). However, the most reactive derivative was compound IX, which lacks C-4 substituent (Table I). This compound becomes 1.75 times more reactive than compound II and 11.7 times than compound VIII (Table I).

As can be seen from Fig. 1, the nitrosoaryl derivatives could react with alkylperoxyl radicals through two different groups, i.e. the nitroso group and the dihydropyridine ring. After analyzing Table I, we concluded that the contribution to the reactivity of the nitroso group is more relevant than that of the dihydropyridine ring as is supported by the values of the corresponding kinetic rate constants of the nitrosoaryl and nitroaryl derivatives (Table I). On the other hand, the position either —NO or $—NO_2$ group in the aryl moiety did not significantly affect the reactivity of the corresponding 1,4-DHP derivatives towards the tested alkylperoxyl radicals.

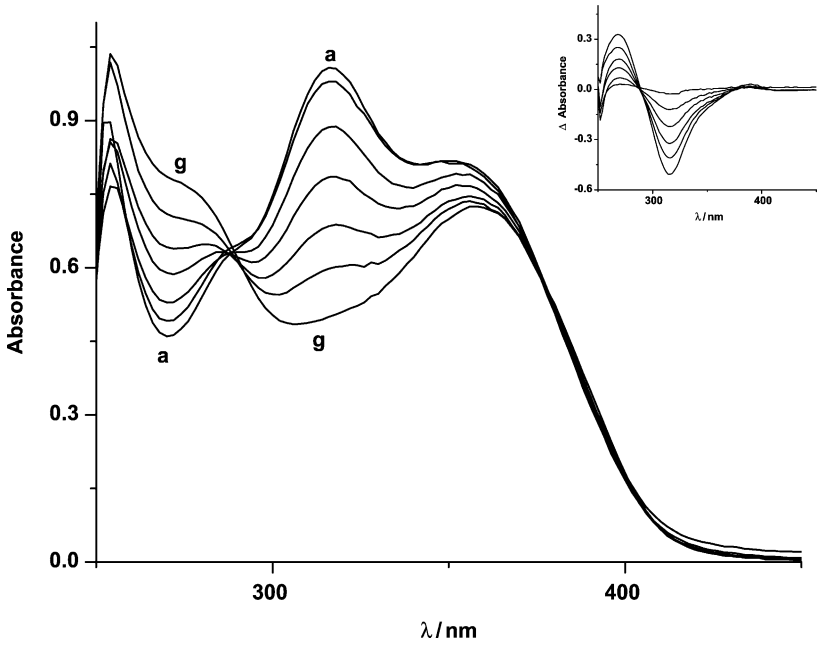


FIGURE 3 UV-Vis spectra corresponding to reaction between a 100 μM compound V solution and 20 mM ABAP-derived alkylperoxyl radicals solution in 0.04 M Britton–Robinson buffer/DMF 70/30 at pH 7.4. a–f: 60 min. Insert: differential UV-Vis spectra corresponding to reaction between a 100 μM compound V solution and 20 mM ABAP-derived alkylperoxyl radicals solution in 0.04 M Britton–Robinson buffer/DMF 70/30 at pH 7.4.

TABLE I Kinetic rate constants of DHP derivates for the reaction with ABAP-derived alkylperoxyl radicals in 0.04 M Britton–Robinson buffer/DMF (70/30) at pH 7.4 and 37°C

Compound	$k(M^{-1}\,s^{-1})$*	Rel. Trolox†	Rel. nisoldipine‡	Ep(mV)¶
Nitrosoaryl 1,4-DHP				
I	6357 ± 98	1.3	6.6	748
II	6510 ± 215	1.3	6.8	740
III	6062 ± 170	1.2	6.3	761
IV	5997 ± 32	1.2	6.3	761
V	5991 ± 156	1.2	6.2	742
Nitroaryl 1,4-DHP				
VI	861 ± 31	0.2	0.9	753
VII	939 ± 19	0.2	1.0	764
VIII	972 ± 12	0.2	1.0	777
Miscellaneous				
IX	11,410 ± 390	2.3	11.9	352
X	2165 ± 57	0.4	2.2	715
XI	960 ± 50	0.2	1.0	681
XII	4920	1.0	5.0	236

*Kinetic rate constants were calculated by using the rate constant for the reaction between Trolox and alkylperoxyl radicals ($k = 4.92 \times 10^3\,M^{-1}\,s^{-1}$, taken from Ref. [21]). †Ratio between kinetic rate constants of the tested DHP derivatives/kinetic rate constant of Trolox in presence of alkylperoxyl radicals. ‡Ratio between kinetic rate constants of the tested DHP derivatives/kinetic rate constant of Nisoldipine in presence of alkylperoxyl radicals. ¶Oxidation peak potential values determined by DPV using a glassy carbon working electrode in aqueous 0.04 M Britton–Robinson/ethanol (70/30) + 0.1 M KCl at pH 7.4. Potentials are expressed versus Ag/AgCl reference electrode. 1,4-DHP concentration: 0.5 mM.

Also, the ester groups in 3- and 5-position did not affect the reactivity of the assayed compounds.

The reactivity of the nitrosoaryl 1,4-DHP derivatives (Compounds I–V) was higher than that of Trolox (Table I). However, Trolox was 5 times more reactive than the nitroaryl 1,4-DHP derivatives (Compounds VI–VIII).

Compound X (Fig. 1) containing a phenyl group in C-4 position was 2.2 times more reactive than compound VIII, which had a 4-nitrophenyl group in C-4 position and 2.8 times less reactive than compound IV, having a 4-nitrosophenyl group in C-4 position. These results support the contention that the nitro group diminished the reactive of the compounds in contrast with the presence of the nitroso group in the molecule.

Nitroaryl 1,4-DHP derivatives showed a comparable reactivity against a well-known antioxidant 1,4-DHP drug, such as, nisoldipine (Table I). In contrast, nitrosoaryl 1,4-DHP (Compounds I–V) and compound IX were significantly more reactive than nisoldipine (Table I).

From Table I, it is apparent that the oxidation peak potential values of both nitrosoaryl and nitroaryl 1,4-DHP derivatives exhibited close values (680–770 mV), therefore the differences in reactivity could be ascribed to the presence of nitroso group in the aryl moiety. However, compound IX (C-4 unsubstituted dihydropyridine) and Trolox, have oxidation peak potential values significantly lower than those of the remaining compounds, i.e. easier oxidation under these experimental conditions. This factor could explain the enhanced reactivity for compound IX compared with the resting 1,4-DHP.

On the other hand, in spite of the proximity of the oxidation potential values between Trolox and the 1,4-dihydropyridine IX, a substantial difference in reactivity was found (Table I).

Additional experiments permitted us to conclude that for 4 h, all the tested 1,4-DHP were consumed by free radicals, since the formed pyridine derivative remained stable at least during the following 3–4 h of the experiment. In this time-scale, the concentration of alkylperoxyl radicals was practically constant, indicating that the pyridine derivative was unable to react with alkylperoxyl radicals.

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 various degrees. The addition of different 1,4-DHP concentrations to a colored solution of ABTS radical cation produced a diminution of the characteristic absorbance at 734 nm corresponding to the radical cation. Figure 4 plots the time-course absorbance at 734 nm in the presence of two different concentrations of compound II. This plot shows a concentration-dependent effect, thus the bleaching ability of 1,4-DHP depends on the concentration of the drug. To assess the kinetic rate constants of the 1,4-DHP, the slopes of time-course of the concentration of ABTS radical cation in the presence of different 1,4-DHP concentration were used.

Table II exhibits the reactivity of the different tested compounds towards ABTS radical cation and their relative reactivity with nisoldipine. In this table, the nitrosoaryl 1,4-DHP derivatives were the most reactive compounds. Compound III (nitroso derivative) was 15 times more reactive than compound VIII (nitro derivative). Likewise, as occurred with

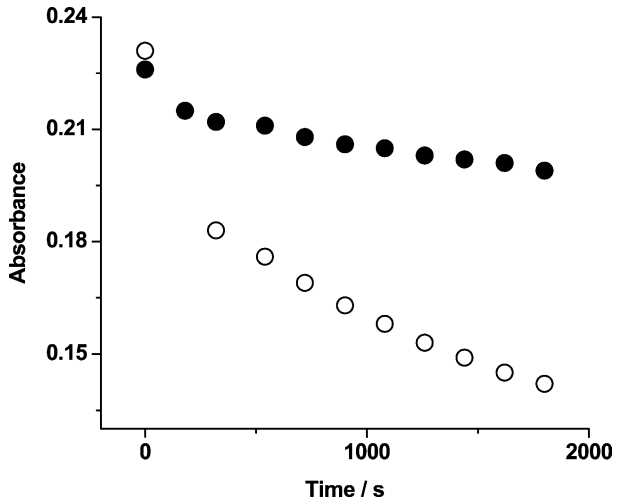


FIGURE 4 Time-course of the absorptivity at 734 nm of 15 μM ABTS radical cation solution in the presence of different concentrations of compound II in 0.04 M Britton–Robinson buffer/ethanol 70/30 at pH 7.4 and 15°C. (●) 1μM and (○) 5 μM.

alkylperoxyl radicals, compound IX was the most reactive 1,4-DHP (Table II). However, in this case Trolox resulted the most reactive compound, being 1.3 times more reactive than compound IX (Table II).

On the other hand, the position either —NO or $-NO_2$ group in the aryl moiety did not significantly affect the reactivity of the corresponding 1,4-DHP towards the tested ABTS radical cation. Also, the ester groups in 3- and 5-position did not affect the reactivity of the assayed compounds.

Studies on the Reactivity towards Alkylperoxyl Radicals followed by the GC/MS Technique

In order to identify the products obtained after the reaction between 1,4-DHP derivatives and the free radicals, the GC/MS technique was used (Tables III and IV). Some conclusions on these studies can be summarized as follows: (a) the GC/MS procedure used to characterize the parent 1,4-dihydropyridines and its subsequent reactivity with radicals did not require a derivatization process; (b) the tested nitrosoaryl 1,4-DHP derivatives reacted with alkylperoxyl radicals, forming a nitro-pyridine derivative as a final product; (c) the nitroaryl 1,4-DHP derivatives reacted with alkylperoxyl radicals, forming a nitro-pyridine derivative as a final product; (d) the retention times of parent nitrosoaryl 1,4-DHP were smaller than their corresponding nitroaryl 1,4-DHP derivatives. Furthermore, the fragmentation pattern for the nitrosoaryl 1,4-DHP was more significantly different than that for the nitroaryl 1,4-DHP; (e) the parent nitroso group was not detected after the reaction with alkylperoxyl radicals, indicating that the transformation to nitroaryl was complete under our experimental conditions; (f) 1,4-dihydropyridines after the reaction with alkylperoxyl radicals suffered a dehydrogenation process of the 1,4-DHP ring,

TABLE III Proposed base peaks (Bp) and the molecular ion fragments of parent 1,4-DHP in 0.04 M Britton–Robinson/DMF (70/30) at pH 7.4 and 25° C

Derivative	Base peak	M^+	R_t(min)
I	280 M^+—$PhNO_2$	402	12.2
II	280 M^+—$PhNO_2$	402	12.2
III	252 M^+—$PhNO_2$	374	12.7
IV	224 M^+—$PhNO_2$	346	12.3
V	252 M^+—$PhNO_2$	374	12.2
VI	224 M^+—$PhNO_2$	346	11.8
VII	224 M^+—$PhNO_2$	346	11.7
VIII	224 M^+—$PhNO_2$	346	12.3
IX	224 M^+—Oet	253	7.7
X	224 M^+—Ph	301	12.2

TABLE II Kinetic rate constants of DHP derivatives for the reaction with ABTS radical cation in 0.04 M Britton–Robinson buffer/ethanol (70/30) at pH 7.4 and 15°C

Compound	$k(M^{-1}s^{-1})$*	Rel. Trolox†	Rel. nisoldipine‡
Nitrosoaryl 1,4-DHP			
I	577 ± 10	0.14	11.3
II	419 ± 2	0.10	8.2
III	593 ± 20	0.14	11.6
IV	400 ± 3	0.10	7.8
V	425 ± 5	0.10	8.3
Nitroaryl 1,4-DHP			
VI	19 ± 2	0.005	0.4
VII	30 ± 2	0.007	0.6
VIII	40 ± 2	0.001	0.8
Miscellaneous			
IX	3213 ± 165	0.8	62.7
X	194 ± 10	0.05	3.8
XI	51 ± 2	0.01	1
XII	4100 ± 10	1.0	80.4

*Kinetic rate constants were calculated from the $ABTS^{\cdot+}$ concentration/time plots. †Ratio between kinetic rate constant of the tested DHP derivates/kinetic rate constant of Trolox. ‡Ratio between kinetic rate constant of the tested DHP derivates/kinetic rate constant of Nisoldipine.

TABLE IV Proposed base peaks (Bp) and the molecular ion fragments of oxidized derivatives obtained after the reaction with alkylperoxyl radicals in 0.04 M Britton–Robinson/DMF (70/30) at pH 7.4 and 25°C

Derivative	Base peak	M^+	R_t(min)	% Conv.* NO → NO_2	% Conv.† DHP → Py
I	298 M^+—COOipr − Me	400	11.0	100	27.2
II	298 M^+—COOipr − Me	400	11.0	100	23.1
III	327 M^+—NO_2	372	10.7	100	28.9
IV	313 M^+—OMe	344	9.9	100	32.9
V	327 M^+—NO_2	372	10.6	100	38.5
VI	298 M^+—NO_2	344	9.5	–	56.6
VII	327 M^+—O	344	9.9	–	56.3
VIII	313 M^+—OMe	344	10.0	–	42.8
IX	206 M^+—OEt	251	6.0	–	100
X	236 M^+—OMe × 2	299	11.0	–	51.9

*Percentage of conversion parent nitrosoaryl derivative to nitroaryl 1,4-DHP after the reaction with radicals. †Percentage of conversion dihydropyridine moiety to the corresponding pyridine derivative after the reaction with radicals.

yielding pyridine-derived metabolites at different grades. This conclusion is supported by the respective retention times and the mass fragmentation pattern for each compound is displayed in Table IV. Clearly, an electron transfer reaction is involved in the scavenging of the free radicals; (g) by comparison of the retention times corresponding to the pyridine derivatives and the parent compounds, the former resulted lower. This result could be explained on the basis of the varied affinity for the stationary phase, since that pyridine derivatives had lost an hydrogen in 1-position in the dihydropyridine ring and the loss of a second hydrogen by the formation of an aromatic ring; (h) the mass fragmentation pattern of the aromatized derivatives shows a different peak base depending on 4-substitution on the 1,4-dihydropyridine ring.

Figure 5 shows ion chromatograms for 45 min of reaction between 100 μM compound V and ABAP-derived alkylperoxyl radicals in 0.04 M Britton–Robinson/DMF buffer (70/30) at pH 7.4. As can be seen from this figure, at this time of reaction four compounds coexisted, i.e. nitroso 1,4-dihydropyridine, nitro 1,4-dihydropyridine, nitrosopyridine and nitro-pyridine. A most detailed time-course for this reaction is presented in Table V. At a short time of reaction (30 min), practically all the nitroso 1,4-DHP derivatives were oxidized to the corresponding nitro 1,4-DHP, however, the oxidation of the dihydropyridine to the corresponding pyridine derivative began to be important after 90 min of reaction (12% nitropyridine). At 2 h of reaction (Table V), only two compounds were predominantly found, i.e. nitro 1,4-dihydropyridine (61%) and nitro-pyridine (38.5%).

Also, the time-course of the reaction between alkylperoxyl radicals and 1,4-dihydropyrines (100 μM compound V) was followed by DPV using a glassy carbon electrode as working electrode (Fig. 6). As can be seen from this figure, by means of this electrochemical technique, the differentiation between the nitroso group (−120 mV) and the nitro group (−670 mV) was at least possible, confirming that as a consequence of the reaction between 1,4-DHP and alkylperoxyl radicals, most nitroso derivatives were oxidized to the corresponding nitro derivative.

Studies on Reactivity of 1,4-Dihydropyridines with Alkylperoxyl Radicals Conducted by HPLC Technique with the PDA Detector

These studies were conducted to evaluate final products of the reaction between 1,4-DHP derivatives and alkylperoxyl radicals. Under our experimental conditions and the selected mobile phases the developed HPLC method permitted the assessment of both parent 1,4-DHP derivatives and their corresponding oxidized products (pyridine derivatives) during the reaction as shown in Fig. 7.

In general, retention times for parent compounds varied from 9 to 14 min and their respective oxidized products appeared at shortest times, i.e. 7–12 min. However, the unsubstituted 1,4-dihydropyridine (compound IX) exhibited a retention time of 3 min. The relative standard deviation of the retention time was $<1\%$, indicating high stability of the system.

Figure 7 shows a typical HPLC chromatograms for compound V (Fig. 7A) and compound VIII (Fig. 7B) after the reaction with alkylperoxyl radicals. As can be seen from this figure, the oxidized products appear at shorter retention times than those of the parent compounds. Thus, in Fig. 7A, the parent nitrosoaryl 1,4-DHP derivative appears at 11.2 min, but the corresponding pyridine derivative appeared at 10.3 min. Likewise, the nitroaryl 1,4-DHP (compound VIII, Fig. 7B) had a retention time of 14.3 min and the pyridine had a retention time of 11.6 min.

On the other hand, the area under curve (AUC) values corresponding to the pyridine derivatives were similar to those found for GC/MS chromatography (Table IV), i.e. AUC values ranging from 20 to 55%, with the exception of compound IX exhibiting a 100% oxidation.

In conclusion, the main advantage of this method resides in the simultaneous determination of both

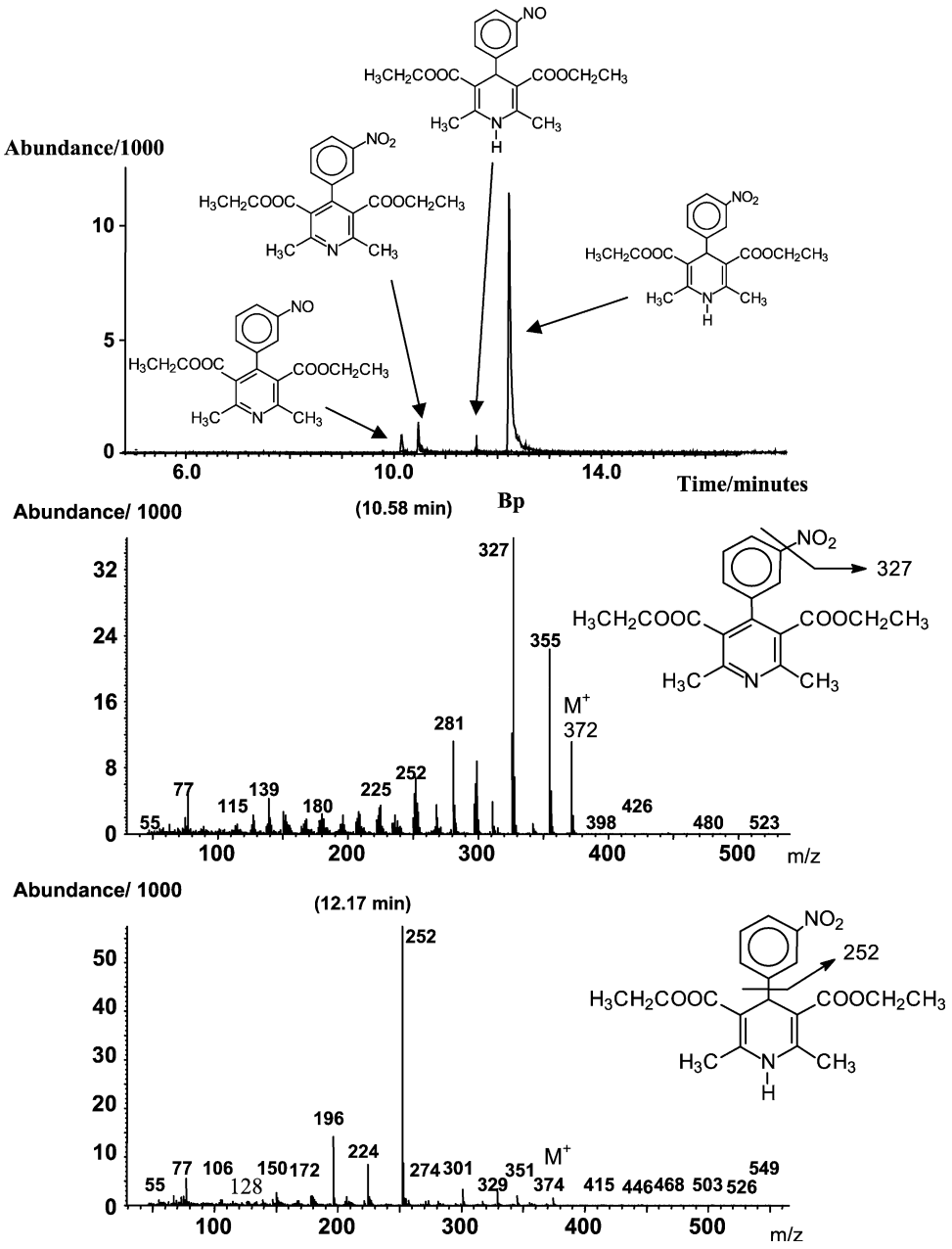


FIGURE 5 Extracted ion chromatograms and mass spectrum of 100 μM solution of compound V after 45 min of reaction with alkylperoxyl radicals in 0.04 M Britton–Robinson buffer/DMF 70/30 at pH 7.4.

the parent 1,4-DHP derivative and its oxidation product generated in the course of the reaction between 1,4-DHP derivatives and alkylperoxyl radicals, confirming that an electron transfer reaction occurred during this process.

Studies on Reactivity of Nitrosopyridines and Nitropyridines Electrolytically Generated with Alkylperoxyl Radicals

To deepen into the mechanism of reaction, some experiments were performed with CPE to oxidize the 1,4-DHP derivatives. On the other hand, DPV was used to follow concentration changes of the parent 1,4-DHP during electrolysis.

To investigate the particular preponderance of chemical groups in the 1,4-DHP molecule with the ability to react with alkylperoxyl radicals, aqueous solutions at pH 7.4 were electrolyzed between +950 and +1000 mV on a reticulate glassy carbon electrode to oxidize the dihydropyridine ring. As can be seen from Fig. 8, when an oxidation potential was applied to either nitrosoaryl (compound V) or nitroaryl derivative (compound VII), a decrease in the oxidation signal occurred (Fig. 8). But, under these electrolytic conditions, the currents corresponding

TABLE V Percentage abundance corresponding to the base peaks (Bp) of both parent and oxidized derivatives during the reaction between 100 μM compound V and 20 mM ABAP-derived alkylperoxyl radicals in 0.04 M Britton–Robinson/DMF (70/30) at pH 7.4 and 25°C

Time (minutes)	% Ion 252 nitroso-DHP $R_t = 11.8$ min	% Ion 252 nitro-DHP $R_t = 12.2$ min	% Ion 342 nitroso-Py $R_t = 10.1$ min	% Ion 372 nitro-Py $R_t = 10.6$ min
0	100	0	0	0
5	61.9	38.1	0	0
10	40.3	59.7	0	0
15	32.7	64.5	2	0.8
20	24.9	68.2	6	0.9
25	14.6	77.8	6.9	0.7
30	6.6	85.5	6.2	1.7
35	4.4	89.9	3.9	1.8
45	2.0	92.0	2.5	3.5
55	0.2	93.6	2.2	4.0
90	0	86.4	1.3	12.3
120	0	61	0.2	38.8

to the reduction signals remained unaltered. This means that both the nitroso- and nitro-group were unaffected by the electro-oxidation of the molecule. Thus, after the complete electrolysis, the nitrosopyridine derivative and nitropyridine derivative were found as the final products of the oxidation process (followed by GC/MS and HPLC-PDA), for nitrosoaryl 1,4-DHP and nitroaryl 1,4-DHP, respectively.

The final products of the electrolysis (nitrosopyridine and nitropyridine) were used for the reaction with the alkylperoxyl radicals at 37°C and pH 7.4. The time-course of the reactions were followed by UV-Vis spectroscopy at $\lambda = 318$ and at 354 nm for nitrosoaryl 1,4-DHP and nitroaryl 1,4-DHP, respectively.

The addition of the free radical to the nitrosopyridine derivative solution produced a decrease of absorptivity at $\lambda = 318$ nm with time. This decay means that the nitroso group reacted towards the alkylperoxyl radical. In contrast, the absorptivity of nitropyridine derivative corresponding to compound VII did not change after the addition of the alkylperoxyl radical to the reaction mixture, at least in the time-scale of the experiment (2 h). These results support the view that nitrosoaryl 1,4-DHP derivatives (compound I–V) exhibited two reactive centers, i.e. the nitroso group and the dihydropyridine ring. The nitroaryl 1,4-DHP derivatives (compounds VI–VIII) present a single reactive center, i.e. the dihydropyridine ring.

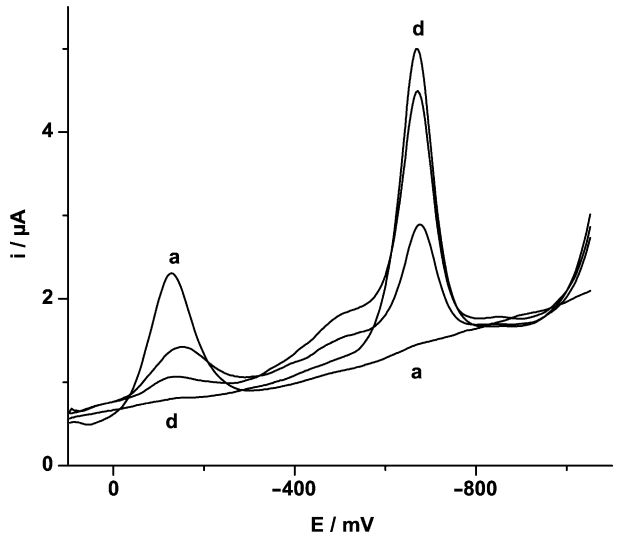


FIGURE 6 Time-course of the reaction between 100 μM compound V and 20 mM alkylperoxyl ABAP-derived radicals followed by DPV. $a = 0$ min, $d = 90$ min.

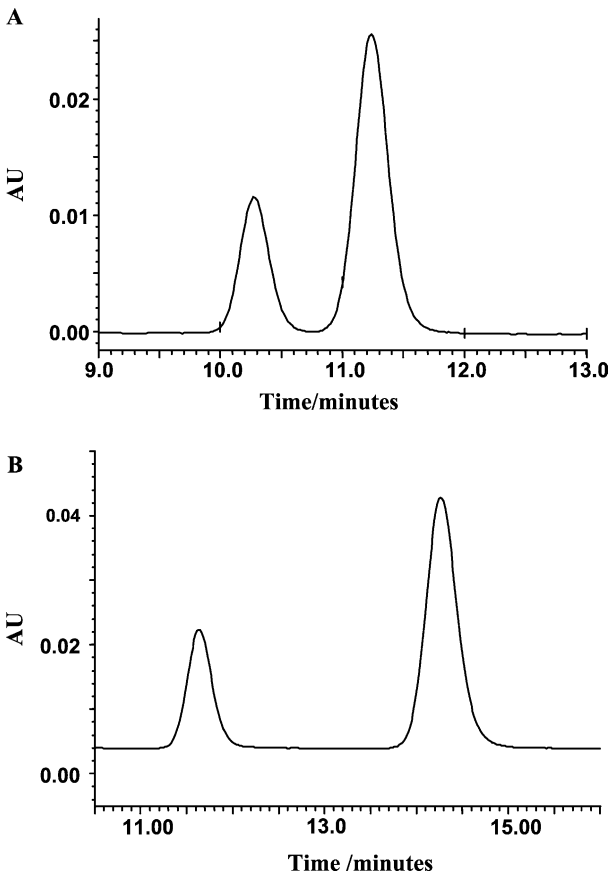


FIGURE 7 Typical HPLC-PDA chromatograms for the reaction between 20 mM alkylperoxyl radicals with: (A) 100 μM compound V and (B) 100 μM compound VIII.

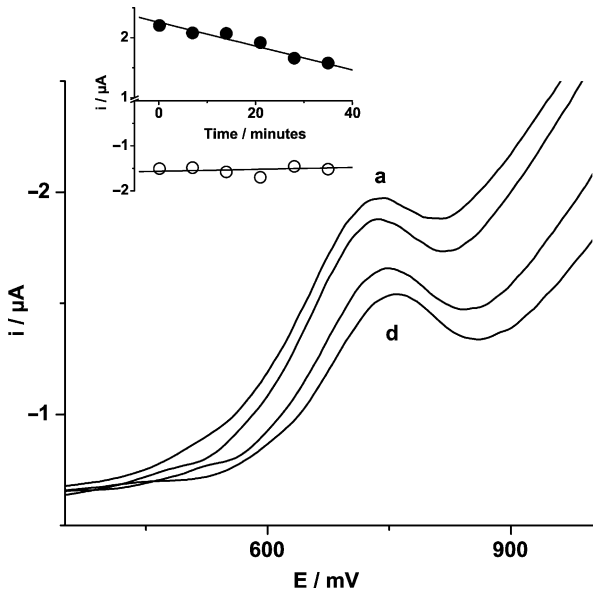


FIGURE 8 Time-course of CPE at +950 mV of 100 µM compound V in aqueous media followed by DPV. a–d: 1 h. Insert: evolution of nitroso group peak current (○) and evolution of dihydropyridine peak current (●).

Reaction Mechanisms

Based on the present results obtained by using chromatographic, electrochemical and spectroscopic techniques, the following conclusions can be depicted.

(a) 1,4-DHP derivatives containing a nitroso group, firstly reacted with alkylperoxyl radicals through this chemical group, giving the corresponding nitro derivative (Fig. 9). This finding is consistent with our studies about the time-course of this reaction followed by both GC/MS and DPV (Figs. 5 and 6, Table V). On the other hand, Zuman[24] has previously described that in the oxidation of nitrosobenzenes to corresponding nitro compounds can be achieved with numerous chemical agents. Furthermore, the conversion of nitrosobenzene to nitrobenzene by *tert*-butyl-peroxide radicals has also been reported.[25] On the other hand, the spin-trap properties of the nitroso group have been well-documented.[14,15] Secondly, after the oxidation of the nitroso group to the nitro group, the dihydropyridine ring was partially oxidized to the corresponding pyridine derivative as shown in Fig. 9B.

(b) In the case of nitroaryl 1,4-DHP derivatives, solely the 1,4-DHP ring would react with alkylperoxyl radicals (Fig. 9B), accounting for the differences in the kinetic rate constant values.

(c) From the GC/MS experiments, we concluded that for the nitrosoaryl 1,4-DHP derivatives (compounds I–V), its transformation to the nitroaryl 1,4-DHP derivative was apparently complete after the reaction with alkylperoxyl radicals. In contrast, in the case of the nitroaryl 1,4-DHP derivatives (compounds VI–VIII), the oxidation to the pyridine derivative accounted only for a 30–50% of the initial concentration after the reaction with the alkylperoxyl radicals.

CONCLUDING REMARKS

1. In the present paper, a direct quenching of radical species by a number of synthesized nitrosoaryl 1,4-DHP derivatives and their parent nitroaryl 1,4-DHP was determined. These two series of compounds were compared with the C-4 unsubstituted 1,4-DHP derivative and the corresponding C-4 aryl substituted 1,4-DHP derivative.
2. In general terms, nitrosoaryl 1,4-DHP were more reactive than the corresponding nitroaryl derivatives towards alkylperoxyl radicals and ABTS radical cation. However, the C-4 unsubstituted 1,4-DHP derivative was the most potent agent, being 1.8 and 11.8 fold more reactive than nitrosoaryl 1,4-DHP and nitroaryl 1,4-DHP derivatives, respectively.

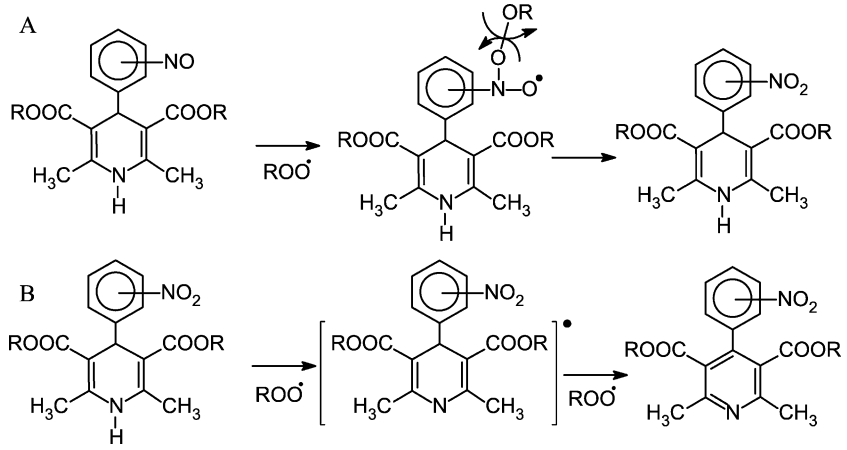


FIGURE 9 Pathway of: (A) Nitrosoaryl 1,4-DHP derivatives after the reaction with alkylperoxyl radicals (B) Nitroaryl 1,4-DHP derivatives after the reaction with alkylperoxyl radicals. Reaction Media: 0.04 M Britton–Robinson buffer/DMF (70/30) at pH 7.4.

3. The position either —NO or —NO_2 group in the aryl moiety did not significantly affect the reactivity of the corresponding 1,4-DHP towards the tested free radicals. Also, the ester groups in 3- and 5-position did not affect the reactivity of the assayed compounds.
4. The pyridine derivative was detected and quantitatively determined as a final product of the reaction with both nitroso- and nitroaryl 1,4-DHP after the reaction with alkylperoxyl radicals. Additionally, we found that the nitrosoaryl 1,4-DHP derivatives generated the corresponding nitropyridine derivative as was assessed by GC/MS technique.
5. Our results strongly support the assumption that the reactivity between the synthesized 1,4-DHP derivatives with alkylperoxyl radicals involves electron transfer reactions, which is documented by the presence of pyridine as product of reaction and the complete oxidation of the nitroso group to the nitro group in the case of the nitrosoaryl 1,4-DHP derivatives.
6. The present results support that the Ca^{+2} channel antagonists of 1,4-dihydropyridines type can behave as lipid antioxidants, a property that may contribute to their role in atherosclerosis and other diseases, including inflammatory vascular diseases and central nervous system trauma and stroke. The antioxidant mechanism of action of 1,4-DHPs depends on the dihydropyridine ring, which can donate electrons to the propagating radicals to reduce it to a non-reactive form.

Acknowledgements

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References

[1] Godfrain, T. (1987) "Classification of calcium antagonist", *Am. J. Cardiol.* **59**, 11B–23B.
[2] Wenzel, R.R., Bruck, H., Noll, G., Daul, A.E. and Philip, T. (2000) "Antihypertensive drugs and sympathetic nervous system", *J. Cardiovasc. Pharmacol.* **35**, S43–S52.
[3] Berkels, R., Roesen, R., Dhein, S., Fricke, U. and Klaus, W. (1999) "Dihydropyridine calcium antagonist-induced modulation of endothelial function: a review", *Cardiovasc. Drug Rev.* **17**(2), 179–186.
[4] Salameh, A., Schomecker, G., Breitkopf, K., Dhein, S. and Klaus, W. (1996) "The effect of calcium-antagonist nitrendipine on intracellular calcium in endothelial cells", *Br. J. Pharmacol.* **118**, 1899–1904.
[5] Zhu, X.-Q., Liu, Y.-Ch. and Cheng, J.-P. (1999) "Which hydrogen atom is first transferred in the NAD(P)H model Hantzsch ester mediated reactions via one-step and multistep hydride transfer", *J. Org. Chem.* **64**, 8980–8981.
[6] Cheng, J.P., Lu, Y., Zhu, X.Q., Sun, Y., Bi, F. and He, J. (2000) "Heterolytic and homolytic N—H bond dissociation energies of 4-substituted Hantzsch 2,6-dimethyl-1,4-dihydropyridines and the effect of one-electron transfer on the N—H bond activation", *J. Org. Chem.* **65**, 3853–3857.
[7] Tong Mak, I., Boehme, P. and Weglicki, W.B. (1995) "Protective effects of calcium channel blockers against free radical-impaired endothelial cell proliferation", *Biochem. Pharmacol.* **50**, 1531–1534.
[8] Velena, A., Zilbers, J. and Duburs, G. (1999) "Derivatives of 1,4-dihydropyridines as modulators of ascorbate-induced lipid peroxidation and high-amplitude swelling of mitochondria, caused by ascorbate, sodium linoleate and sodium pyrophosphate", *Cell Biochem. Funct.* **17**, 237–252.
[9] Mason, R.P., Tong Mak, I., Trumbore, M.W. and Mason, P.E. (1999) "Antioxidant properties of calcium antagonists related to membrane biophysical interactions", *Am. J. Cardiol.* **84**, 16L–22L.
[10] Cominacine, L., Pasini, A.F., Garbin, U., Pastorino, A.M., Davoli, A., Nava, C., Campagnola, M., Rossato, P. and Lo Cascio, V. (2003) "Antioxidant activity of different dihydropyridines", *Biochem. Biophys. Res. Commun.* **302**, 679–684.
[11] López-Alarcón, C., Navarrete, P., Camargo, C., Squella, J.A. and Núñez-Vergara, L.J. (2003) "Reactivity of 1,4-dihydropyridines toward alkyl, alkylperoxyl radicals and ABTS radical cation", *Chem. Res. Toxicol.* **16**, 208–215.
[12] Squella, J.A. and Núñez-Vergara, L.J. (1990) "Polarography as a technique for determining photodegradation in calcium antagonists", *Bioelectrochem. Bioenerg.* **23**, 161–166.
[13] Squella, J.A., Barnafi, E., Perna, S. and Núñez-Vergara, L.J. (1989) "Nifedipine: differential pulse polarography and photodecomposition", *Talanta* **36**, 363–366.
[14] Misik, V., Stasko, A., Gergel, D. and Ondrias, K. (1994) "Spin-trapping and antioxidant properties of illuminated and non-illuminated nifedipine and nimodipine in heart homogenate and model system", *Mol. Pharmacol.* **40**, 435–439.
[15] Ondrias, K., Misik, V., Vergel, D. and Stasko, A. (1989) "Lipid peroxidation of phosphatidylcholine liposomes depressed by the calcium channel blockers nifedipine and verapamil and by the antiarrythmic-antihypoxic drug stobadine", *Biochim. Biophys. Acta* **1003**, 238–245.
[16] Díaz-Araya, G., Godoy, L., Naranjo, L., Squella, J.A., Letelier, M.E. and Núñez-Vergara, L.J. (1998) "Antioxidants effects of 1,4-dihyropyridine and Nitroso aryl derivatives on the Fe^{+3}/Ascorbate-stimulated lipid peroxidation in rat brain slices", *Gen. Pharm.* **31**, 385–391.
[17] Berson, J. and Brown, E. (1955) "Studies on dihydropyridines. I. The preparation of unsymetrical 4-aryl-1,4-dihydropyridines by the Hantzsch-Beyer synthesis", *J. Am. Chem. Soc.* **77**, 444–447.
[18] Stout, D.M. and Meyers, A.I. (1982) "Recent advances in the chemistry of dihydropyridines", *Chem. Rev.* **82**, 223–243.
[19] Halliwell, B. and Gutteridge, J.M.C. (2000) *Free Radicals in Biology and Medicine* (Oxford University Press, Oxford), pp 69–70.
[20] Niki, E. (1990) "Free radical initiators as source of water- or lipid-soluble peroxyl radicals", In: Glazer, A., ed., *Methods in Enzymology* (Academic Press, San Diego), pp 100–108.
[21] Ross, L. and Barclay, C. (1988) "The cooperative antioxidant role of glutathione with to lipid-soluble and water-soluble antioxidant during peroxidation of liposome's initiated in the aqueous phase and in the lipid phase", *J. Biol. Chem.* **263**, 16138–16142.
[22] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999) "Antioxidant activity applying an improved ABTS radical cation decolorization assay", *Free Radic. Biol. Med.* **26**, 1231–1237.
[23] Labudzinska, A. and Gorczynska, K. (1995) "The UV difference spectra as a characteristic feature of phenols and aromatic amines", *J. Mol. Struct.* **349**, 469–472.
[24] Zuman, P. and Shah, B. (1994) "Addition, reduction and oxidation reactions of nitrosobenzene", *Chem. Rev.* **94**, 1621–1641.
[25] Johnson, N.A. and Gould, E.S. (1973) "Metal ion catalysis of oxygen transfer reactions. III. Transition metal chelates as catalysts in the oxidation of nitrosobenzene. Oxygen transfer from alkyl peroxy radicals", *J. Am. Chem. Soc.* **95**, 5198–5204.