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Arylated α- and β-dihydrofuran naphthoquinones: Electrochemical parameters, evaluation of antitumor activity and their correlation

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ABSTRACT

We herein report the antitumor activity of several substituted α- and β-dihydrofuran naphthoquinones against 4 human tumor cell lines, HL-60 (leukemia), SF-295 (CNS), HCT-8 (colon) and MDA-MB435 (melanoma), and their electrochemical parameters, in the absence and presence of oxygen, in comparison with their non-substituted precursors. These compounds were prepared from readily available lawsone and olefins in the presence of cerium (IV) ammonium nitrate. The β-dihydrofuran naphthoquinones were shown to be highly cytotoxic, while their positional α-isomers were considered less active. The level of intracellular ROS release and the first wave redox potentials were also analyzed and compared with the kinetic constants of the reactivity of quinones with oxygen (k_{app}) obtained through cyclic voltammetry. Significantly positive correlations between ROS release and oxygen reactivity were obtained, while IC_{50} *vs.* ROS release; $-E_{plc}$ *vs.* k_{app} or ROS values correlated in an inverse manner, *i.e.*, the less negative the potential, higher the activities. These findings reinforce the effectiveness of the combination of pharmacology and electrochemistry in medicinal chemistry, in the search of lead anticancer compounds.

1. Introduction

Cancer is a generic term for a large group of diseases that can affect any part of the body and is a major public health problem all over the world. According to the World Health Organization, deaths from cancer worldwide are projected to continue rising, with an estimated 13.1 million deaths in 2030 [1]. The pharmaceutical market requires new drugs to fight cancer that are more selective and less toxic to the human body.

Compounds containing the quinone nucleus are important due to their pharmacological properties as microbicide, trypanocide, viruscide, antifungal and antitumor compounds [2–11]. Several clinically important anticancer drugs such as daunorubicin (**1**), doxorubicin (**2**), and mitomycin C (**3**) contain the quinone moiety as a relevant part of their structures (Fig. 1). Daunorubicin (**1**) and doxorubicin (**2**) are in widespread clinical use, mainly against leukemias, whereas doxorubicin presents a large spectrum of anticancer activity against a variety of solid tumors as well as acute leukemias [10,11]. In the class of naphthoquinones we can highlight the dihydrofuran naphthoquinones for which there are several reports in the literature [2–9].

The present study focused on the evaluation of the cytotoxic activity toward different human cancer cell lines, such as MDA-MB435 (melanome), HCT-8 (colon), SF-295 (CNS), and HL-60 (leukemia) of several synthetic α- and β-dihydrofuran naphthoquinones (Scheme 1, **5a–g**; **6a**, **6c–f**), in comparison to their precursors, *nor*-α- and β-lapachones, **5** and **6**, respectively [4], along with electrochemical studies. The electrochemical investigation was conducted in aprotic media, in the absence and presence of oxygen, to mimic one of the most important mechanisms

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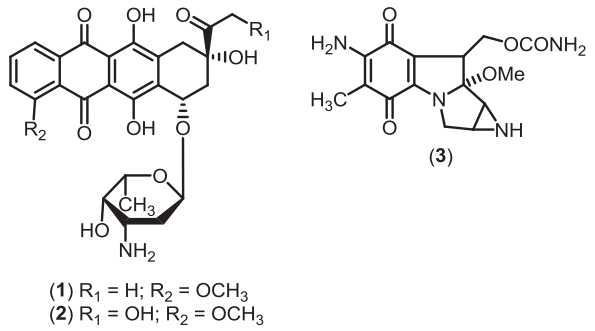


Fig. 1. Structures of daunorubicin (**1**), doxorubicin (**2**) and mitomycin C (**3**).

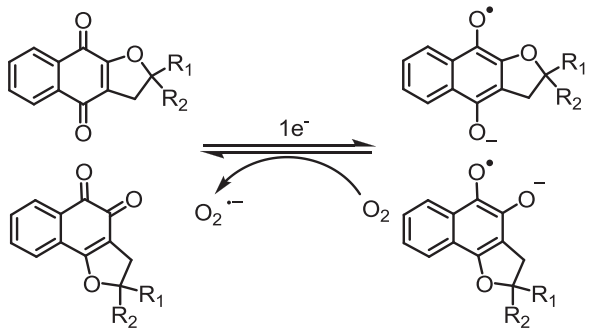


Fig. 2. Proposed redox cycling process for substituted α- and β-dihydrofuran naphthoquinones.

of molecular action of quinones: the generation, after reduction and electron transfer to oxygen, of reactive oxygen species (ROS) [12–20]. The formation of ROS and the redox cycling of quinones may ultimately lead to a cellular condition known as oxidative stress, which can affect cell behavior in many ways [17]. The quinone/semiquinone/hydroquinone ($Q/SQ^{\bullet -}/H_2Q$) triad is an important component of many redox systems in biology. It is a vital link in the electron transfer through cells and tissues [20] (Eqs. (1)–(3)).

$$Q + e^- \rightleftharpoons SQ^{\bullet -} \quad (1)$$

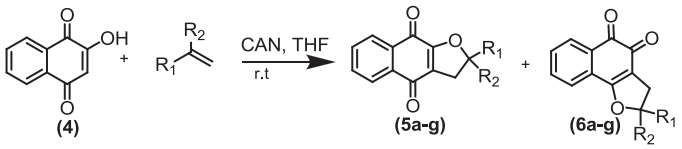
$$SQ^{\bullet -} + e^- + 2H^+ \rightleftharpoons H_2Q \quad (2)$$

$$\text{Net} \quad Q + 2e^- + 2H^+ \rightleftharpoons H_2Q \quad (3)$$

Electrochemical techniques have been extensively used to clarify drugs' mechanism of action [12–16], providing excellent insights into the mode of action of agents, and inspiring further drug design. This approach is particularly suitable for states of the disease associated with oxidative stress of the cells, as in cancer [15–19]. Electrochemistry allows obtaining information on the fundamental thermodynamics and kinetics of the reaction of semiquinone radical $SQ^{\bullet -}$ (generated after a monoelectronic electron transfer) with dioxygen to form the anion radical superoxide [20] (Fig. 2).

The thermodynamics is given by the redox potentials and the second-order rate constants of various semiquinones with dioxygen can be compared with the biological results, concerning ROS release in HL-60 cells.

In organic solvents, like DMF and DMSO, $O_2^{\bullet -}$ is a long-lived species with decrease of disproportionation reactions, making this radical stable even at the time scale of low-scan-rate voltammetry [20–23], so this was the reason for using DMF, as already reported [23–26].



a, R_1 = H, R_2 = C_6H_5
b, R_1 = H, R_2 = p-CH_3-C_6H_5
c, R_1 = H, R_2 = p-Cl-C_6H_5
d, R_1 = H, R_2 = p-F-C_6H_5
e, R_1 = H, R_2 = p-Br-C_6H_5
f, R_1 = CH_3, R_2 = C_6H_5
g, R_1 = H, R_2 = 2,4-diCH_3-C_6H_5
5 (*nor*-α-LP), R_1 = CH_3, R_2 = CH_3
6 (*nor*-β-LP), R_1 = CH_3, R_2 = CH_3

Scheme 1. Synthetic route used for the preparation of α- and β-dihydrofuran naphthoquinones, **5a–g** and **6a–g**, respectively. The compounds **6b** and **6g** have been shown to be unstable and were discarded.

2. Experimental

2.1. Materials and apparatus

Melting points were obtained on a Fischer-Johns apparatus and are uncorrected. Analytical grade solvents were used. Reagents were purchased from Aldrich or Acros Chemical Co. Column chromatography was performed on silica gel 60 (Merck 70-230 mesh). Yields refer to chromatographically and spectroscopically homogeneous materials. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and either an ethanolic solution of cerium sulfate. Infrared spectra were recorded on a Perkin-Elmer FT-IR Spectrum One spectrophotometer, calibrated relative to the 1601.8 cm^{-1} absorbance of polystyrene. NMR spectra were recorded on a Varian Unity Plus VXR (300 MHz) spectrometer in DMSO-d_6 and $CDCl_3$ solutions and solvent or tetramethylsilane was used as the internal standard (δ = 0 ppm), respectively.

2.2. Synthesis of α- and β-dihydrofuran naphthoquinones

The syntheses of the compounds were already reported [4] and followed Scheme 1, which displays the products formed from reaction with lawsone and different styrenes.

In brief, to a round-bottom flask equipped with a magnetic stirring bar, a solution of CAN (1.260 g, 2.3 mmol) in dried THF (10 mL) was added dropwise to an ice-cooled solution of 2-hydroxy-1,4-naphthoquinone (0.174 g, 1 mmol) and diene (2 mmol) in dried THF (10 mL), following a procedure already described [27]. The resulting mixture was stirred for 30 min. Then the mixture was extracted with ethyl acetate and water. The combined organic extracts were washed with water, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuum*. The reaction led to two products: the α-and β-dihydrofuran naphthoquinones which were separated by column chromatography on silica gel, using a gradient of hexane/AcOH as eluent. All the compounds were obtained in good yields and were fully characterized by spectroscopic methods such as infrared (IR), ^1H and ^{13}C nuclear magnetic resonance [4]. Compounds **6b** and **6g** were shown to be unstable and could not be analyzed.

2.3. Electrochemical studies

Cyclic voltammetry (CV) experiments were performed with a conventional undivided three-electrode cell using an Autolab PGSTAT-30 potentiostat (Echo Chemie, Utrecht, The Netherlands) coupled to a microcomputer, interfaced by GPES 4.9 software. Glassy carbon (GC—diameter = 3 mm) as the working electrode, a Pt wire as the counter electrode and the reference electrode an

Ag|AgCl, Cl^- (saturated) were used. The GC electrode was cleaned up by polishing with alumina on a polishing felt (BAS polishing kit). The solvent used in aprotic medium studies was distilled under reduced pressure after stirring with anhydrous copper sulfate. In CV experiments, the scan rate varied from 10 to 500 mV s^{-1}. All experiments were conducted at room temperature (25 ± 2 °C) and purging an inert gas (Argon). To investigate the reactivity of *nor*-α-lapachone and *nor*-β-lapachone and their derivatives toward oxygen, electrochemical reduction in aprotic media (DMF + TBAP 0.1 mol L^{-1}) was performed in the presence and absence of oxygen. Each compound was added to the supporting electrolyte and the solution was deoxygenated with Argon before the measurements by cyclic voltammetry. Oxygen was then bubbled into the cell and its concentration was monitored by a dissolved oxygen meter (Digimed DM-4). Cyclic voltammograms were recorded at different oxygen concentrations. The parameters analyzed were observed anodic shift in the potential of the first reduction wave (E_{plc}) and current increase on the same peak (I_{plc}) [24].

2.4. Pharmacological assays

2.4.1. Hemolytic activity

Membrane disruption was performed in 96-well plates following the method described by Jimenez et al. [28]. Briefly, each well receives 100 μL of 0.85% NaCl solution containing 10 mmol L^{-1} $CaCl_2$ and 100 μL of a 2% suspension of mouse erythrocytes in the same medium. Compounds (**5a**–**g**, **6a**, **c**–**f**) were tested at concentrations ranging from 3.9 to 250 μg/mL. Triton X-100 (Isofar, Brazil) 0.1% (in 0.85% NaCl) was used as a positive control. After incubation for 60 min at room temperature, the plate was centrifuged, and the supernatant was removed and the liberated hemoglobin was measured at 540 nm (DTX 880 Multimode Detector, Beckman Coulter, Inc., Fullerton, CA, USA).

2.4.2. Cell lines and cell cultures

The human tumor cell lines used in this work were HL-60 (leukemia), HCT-8 (colon carcinoma), MDA-MB435 (melanoma) and SF-295 (nervous system glioblastoma) kindly provided by the National Cancer Institute (Bethesda, MD, USA). The cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin at 37 °C in a 5% CO_2 atmosphere.

2.4.3. Cytotoxic assays: MTT assay

The cytotoxicity of all compounds was tested against four tumor cell lines, using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-tetrazolium bromide (MTT) (Sigma–Aldrich Co., St. Louis, MO, USA) reduction assay [29]. For all experiments, cells were plated in 96-well plates (10^5 cells/well for adherent cells or 3×10^5 cells/well for suspended cells in 100 μL of medium). Compounds **5**, **5a**–**g**, **6** and **6a**, **6c**–**f** (5 μg/mL) dissolved in DMSO were added to each well (using the HTS – high-throughput screening – biomek 3000 – Beckman Coulter, Inc., Fullerton, CA, USA) and incubated for 72 h. Control groups received the same amount of DMSO. After 69 h of incubation, the supernatant was replaced by fresh medium containing MTT (0.5 mg/mL). Three hours later, the MTT formazan product was dissolved in 150 μL of DMSO, and absorbance was measured at 595 nm (DTX 880 Multimode Detector, Beckman Coulter, Inc., Fullerton, CA, USA). Doxorubicin (0.009–5 μg/mL) was used as positive control.

2.4.4. Measurement of generation of reactive oxygen species

HL-60 cells (human promyelocytic leukemia line) were used in this experiment and were grown as described in Section 2.4.2. Intracellular reactive oxygen species (ROS) accumulation were monitored, in those cells, after incubation of the quinones, at concentrations of 2 μmol L^{-1}, for 1 h, using 2′,7′-dichlorodihydrofluorescein diacetate (H_2-DCF-DA), which is converted into highly fluorescent dichlorofluorescein (DCF) in the presence of intracellular ROS [30]. At the end of the treatment, cells were loaded with 2′,7′-dichlorodihydrofluorescein diacetate (20 μmol L^{-1}) and incubated at 37 °C for 30 min in the dark. Cells were then harvested, washed and resuspended in PBS and analyzed immediately using flow cytometry with the excitation and emission wavelengths of 490 and 530 nm, respectively.

2.4.5. Statistical analysis

The IC_{50} values for MTT assay were obtained by nonlinear regression using the GRAPHPAD program (Intuitive Software for Science, San Diego, CA) from 3 to 4 independent experiments performed in triplicate. Data are presented as means ± S.D. from at least three independent experiments. Pearson correlation test was conducted to determine the correlations among IC_{50}, ROS release, $-E_{plc}$ (V) and k_{app} (s^{-1}); p-value of <0.05 was regarded as significant. Statistical analysis was performed using SAEG 9.1 (System for Statistical Analysis, MG, Brazil).

3. Results and discussion

3.1. Synthesis

The α- and β-dihydrofuran naphthoquinones were obtained by reacting lawsone (**4**) with styrenes in the presence of ceric ammonium nitrate (CAN) as oxidizing agent and the solvent employed anhydrous THF (Scheme 1) [4]. This methodology was adapted from the work described by Nair et al. [27].

3.2. Pharmacological data

The dihydrofuran naphthoquinones were subjected to evaluation for cancer in different human cancer cell lines, MDA-MB435 (breast), HCT-8 (colon), SF-295 (CNS), HL-60 (leukemia) and tested by MTT method *in vitro*, using doxorubicin (0.5 μmol L^{-1}) as positive control. The MTT assay is a colorimetric analysis that quantifies viable cells indirectly based on the conversion of MTT salt from yellow to a purple coloration, due to formazan formation. This test is used to quantify the cytotoxicity of the compounds against several cell lines yielding the mean inhibitory concentration value (IC_{50}).

Some derivatives were highly active in all cancer cell lines evaluated, or in specific cells (Table 1 and Fig. 3), in accordance to National Cancer Institute (NCI) protocols, where compounds exhibiting IC_{50} values lower than 4 μg/mL are considered active [31]. Analysis presented in Table 1 and Fig. 3 showed that both 1,2-naphthoquinones and 1,4-naphthoquinones have proven to be active against various cell lines with an emphasis on substances **6a**, **6c**–**f** (*ortho* derivatives) which were more active than substances **5a**–**g** (*para*-derivatives) (see Fig. 3). Studies to evaluate the mechanism of action of these compounds are in progress. ROS have been recognized as key molecules, which can selectively modify important endobiotics and thus regulate cellular signaling, including apoptosis [12–19]. A variety of anticancer agents induce apoptosis through the generation of ROS [18,19], especially for this class of quinones, as earlier reported [12,15,16]. So, ROS production was also evaluated in HL-60 cells by flow cytometry using the oxidation sensitive fluorescent dye H_2-DCF-DA after 1 h of incubation and the results are listed in Table 1, column 3. *Nor*-β-lapachone (**6**) stimulated ROS generation, while doxorubicin (Fig. 1) was inactive. It is important to emphasize that doxorubicin is the positive control only for cytotoxic activity, once it is a poor prooxidant and its molecular mechanism of action is not ROS-based, following a different pathway [15]. Its cytotoxic effects are generally related to

Table 1
Cytotoxic activity expressed as IC_{50} in μg/mL (μmol L^{-1}) of *para*-derivatives and *ortho*-derivatives in various cancer cell lines. $E_{plc O_2}$ (in DMF + TBAP 0.1 mol L^{-1}) = −0.811 V (c_{O_2} = 0.21 mg L^{-1}).

Entry	HL-60		SF295	MDA-MB435	HCT-8	Hemolysis (g/mL)	$-E_{plc}$ (V)	k_{app} (s^{-1})
	IC_{50}	ROS (%)						
Dox	0.02 (0.04)	–	0.25 (0.45)	0.47 (0.86)	0.04 (0.07)	>250	nd	nd
5	nd	51.12	nd	nd	nd	nd	0.635	0.56
5a	1.77 (6.41)	64.59	2.87 (10.39)	1.09 (3.95)	3.16 (11.44)	>250	nd	nd
5b	2.95 (10.16)	56.54	3.99 (13.74)	1.98 (6.82)	3.67 (12.64)	>250	0.608	0.47
5c	2.95 (9.51)	62.04	3.99 (12.87)	1.98 (6.38)	3.67 (11,83)	>250	nd	nd
5d	2.12 (7.20)	71.59	2.84 (9.65)	1.39 (4.72)	>5	>250	0.589	0.51
5e	2.12 (5.97)	35.05	2.84 (8.00)	1.39 (3.91)	>5	>250	nd	nd
5f	0.97 (3.34)	81.57	0.96 (3.31)	1.13 (3.89)	2.08 (7.17)	>250	nd	nd
5g	>5	21.73	3.86 (12.69)	2.97 (9.77)	>5	>250	0.618	0.45
6	0.39 (1.75)	71.88	0.07 (0.31)	0.36 (1.58)	0.31 (1.36)	>250	0.635	0.65
6a	0.66 (2.39)	84.66	1.15 (4.16)	0.43 (1.55)	1.28 (4.63)	>250	0.569	1.03
6c	0.46 (1.48)	84.04	0.81 (2.61)	0.50 (1.61)	1.19 (3.82)	>250	0.605	0.66
6d	0.62 (2.10)	93.28	0.78 (2.65)	0.39 (1.33)	1.15 (3.91)	>250	0.590	0.74
6e	0.75 (2.11)	92.76	1.04 (2.93)	0.61 (1.72)	1.11 (3.12)	>250	0.559	0.95
6f	0.41 (1.41)	92.64	0.48 (1.65)	0.33 (1.13)	0.59 (2.03)	>250	0.596	0.56

nd = not determined.

Table 2
Pearson correlations among pharmacological and electrochemical results.

	IC_{50}	ROS	$-E_{plc}$ (V)	k_{app} (s^{-1})
IC_{50}	1.00	−0.9825**	0.6162*	−0.6331*
ROS	−0.9825**	1.00	−0.6752*	0.6178*
$-E_{plc}$ (V)	0.6162*	−0.6752*	1.00	−0.8737**
k_{app} (s^{-1})	−0.6331*	0.6178*	−0.8737**	1.00

* $p < 0.05$.
** $p < 0.01$.

its ability to damage cancer cell DNA, what is a consequence of its interaction and inhibition of DNA topoisomerase II enzyme, inducing double-strand DNA breaks, or also due to direct intercalation into DNA, modifying helical torsion [32]. So, in this case, the comparison in relation to ROS is precluded due to different mechanism of molecular action.

As said, the mechanism of molecular action of those quinones was not established. However, data from the literature of similar compounds [15,16], the values of ROS generation and its correlation with the antitumor activity (see Pearson correlation, Table 2) may suggest that ROS mediate or at least contribute to the cytotoxic activity (Table 1, column 2 *vs.* column 3).

3.3. Electrochemical data

In a typical measurement, cyclic voltammograms (CVs) were first recorded in the absence of oxygen, in order to determine the electrochemical reduction behavior of the compounds and their initial cathodic and anodic peak currents. All the quinones, except **5a**, **5c**, **5e** and **5f**, not investigated, showed a quasi-reversible reduction (Fig. 4) and their main electrochemical parameters are listed in Table 1 (column 8). As examples, Fig. 4 displays the cyclic voltammograms of compounds **5** and **6** with the concentration of 0.1 mmol L^{-1}, in aprotic medium (DMF + TBAP 0.1 mol L^{-1}, at a scan rate of 100 mV s^{-1}). As expected for quinonoid compounds, the overall CV profiles of compounds **5** and **6** are similar to the ones reported for other quinones: two couples of cathodic and anodic peaks, represented by diffusional ($E_{plc} \propto \nu^{1/2}$) quasi-reversible couples, with $E_{plc\mathbf{5}}$ = −0.635 V and $E_{pla\mathbf{5}}$ = −0.562 V and $E_{plc\mathbf{6}}$ = −0.635 V and $E_{pla\mathbf{6}}$ = −0.538 V (Fig. 4). The first pair is related to the anion-radical formation ($SQ^{\bullet -}$ of compounds **5** and **6**, Eq. (1)), and the second pair of peaks is broader and ill-defined, as observed before [9,15], due to possible disproportionation in *ortho*-quinones and comproportionation reactions in *para*-quinones. All the other quinones display similar behavior (figure not shown) and data are displayed in Table 1, column 8.

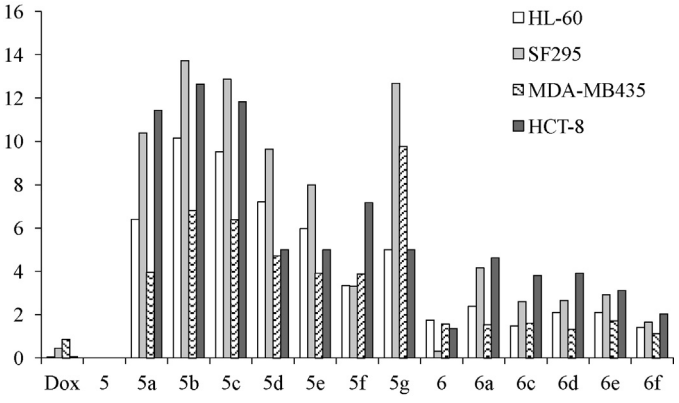


Fig. 3. Cytotoxic activity in μmol L^{-1} for the quinones toward cancer cell lines.

The ease of reduction, established by the comparison among E_{plc} of the compounds is:

6e > 6a > 5d > 6d > 6f > 6c > 5b > 5g > 6 > 5

With the exception of compounds **5d** and **6**, the *ortho*-quinones suffer reduction at less negative potentials, as expected [33], based on physicochemical considerations: electronic asymmetry and polarity of C=O bond, higher in *ortho*-quinones, which make the carbonyl atom of the *ortho* compounds more electron deficient and, hence, easier to reduce [33]. The aryl substituents do not play such an important role in the reduction potential values, since there is no conjugation between the reducible group (the quinone system) and the substituted aryl group. The larger difference between E_{plc} is 76 mV, between **6e** and **5**.

Electrochemical studies also allow the generation of ROS to be evidenced indirectly. Thus, the majority of the compounds, including the precursors were evaluated toward their reactivity with oxygen, in aprotic medium (DMF + TBAP), after monoelectronic reduction (Eqs. (4) and (5)), due to the reasons already appointed [21–26].

$$Q + e^- \rightleftharpoons SQ^{\bullet -} \quad (4)$$

$$SQ^{\bullet -} + O_2 \xrightarrow{kc} Q + O_2^{\bullet -} \quad (5)$$

In Fig. 5, the electrochemical profile for the first reduction wave of compound **6f** is shown. A detailed study of the influence of oxygen concentration on E_{plc} and I_{plc} of the quinones was performed,

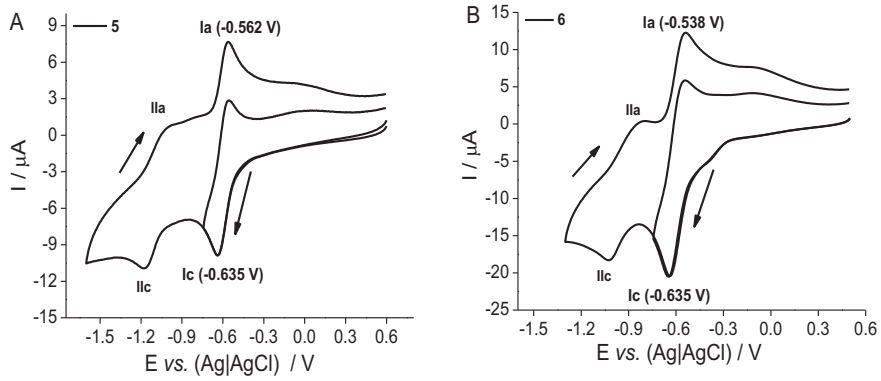


Fig. 4. Cyclic voltammetry in DMF/TBAP (0.1 mol L^{-1}), on GC electrode. Scan rate 100 mV s^{-1}. (A) *Nor*-α-lapachone 1.0 mmol L^{-1} (**5**) and (B) *nor*-β-lapachone 1.0 mmol L^{-1} (**6**).

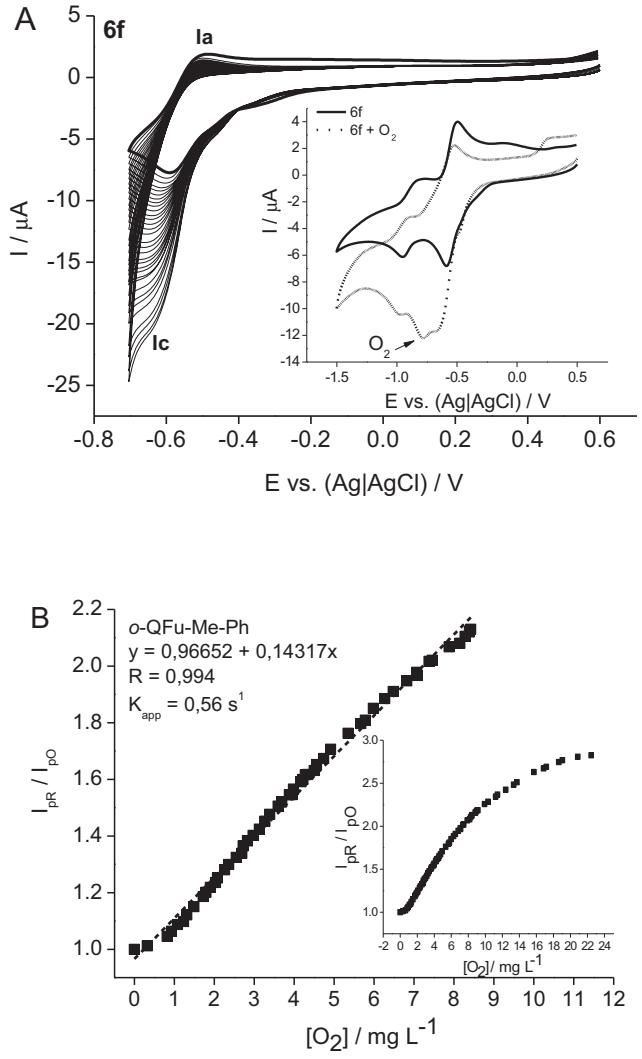


Fig. 5. (A) Cyclic voltammograms for **6f** in DMF/TBAP (0.1 mol L^{-1}), glassy carbon electrode, in the presence of different concentrations of O_2; $\nu = 0.05$ V s^{-1}. Insert: cyclic voltammogram of **6f**, same conditions, in the presence of O_2 (5.0 mg L^{-1}), from 0.5 up to −1.5 V. (B) Linear part of the graph I_{pR}/I_{p0} *vs.* O_2 concentration to obtain the apparent constant of reactivity, k_{app}. Insert: saturation graph for I_{pR}/I_{p0} depending on O_2 concentration.

as described previously [24–26]. The addition of O_2 to the system causes remarkable changes in the position of the first reduction peak potential (E_{pIc}). The peak of oxygen reduction (E_{pO_2}), in this medium, occurs at −0.811 V. These effects include: (a) an increase of the height of the first cathodic wave Ic (Fig. 5a), related to the generation of the semiquinone, being O_2 concentration dependent and (b) disappearance of the corresponding anodic wave Ia (Fig. 5a).

Data obtained from the addition of different concentrations of oxygen (Fig. 5b) allow to determine the apparent association constants (k_{app}) between the electrogenerated semiquinones and O_2 from the graph I_{p1c}/I_{pO1} *vs.* [O_2], based on the equation described by Bard and Faulkner [35]

$$I_{pc} = \frac{I_{pc}}{I_{pO}} = \frac{kRT[O_2]}{nF\nu} \quad (6)$$

where I_{pc} = catalytic peak current; I_{pc}/I_{pO} = current standard; $k[O_2] = k_{app}$ is apparent catalytic constant (s^{-1}); ν = scan rate (V s^{-1}), n = number of electrons, F = Faraday constant (96 485 C mol^{-1}), T = temperature (298 K), and R = 8.314 J mol^{-1} K^{-1}, considering that the maximum solubility of oxygen in DMF is 1.85 mM at 25 °C and are listed in Table 1, column 9.

In this study, using electrochemical methods, we have demonstrated that the anion radicals of the quinones [$SQ^{\bullet -}$] interact with O_2 according to an EC' mechanism, with one heterogeneous (Eq. (4)) and one homogeneous (Eq. (5)) electron transfer steps, yielding the original quinone and the superoxide anion radical [24–26] (Fig. 2 and Eqs. (4) and (5)).

The k_{app} obtained can be related to the capacity of generation of superoxide anion radical. The reactivity order is the following:

6a > 6e > 6d > 6c > 6 > 6f and **5 > 5d > 5b > 5g**

Experiments performed in the presence of oxygen have shown that the majority of the most active cytotoxic quinones, *i.e.*, the *ortho* representatives, react faster with oxygen than the *para*-isomers and provoke a greater release of ROS. These findings corroborate with the ones obtained by flow cytometry (compare columns 3 and 9, in Table 1 and Fig. 6). Table 2 and the graph in Fig. 6 allow the comparison between the kinetics of the reactivity quinone-O_2 (light gray) (Table 1, column 9) and ROS release (dark gray) (Table 1, column 3) in HL-60 cells. The correlation is best evidenced in the next topic, by statistical analysis.

3.4. Statistical results

Correlation between IC_{50}, ROS, $-E_{pIc}$ (V) and k_{app} (s^{-1}) was established (Table 1). A statistically significant negative correlation was observed between IC_{50} and ROS (−0.98) and $-E_{pIc}$ with k_{app} (−0.87) ($p < 0.01$), and also between IC_{50} with k_{app} (−0.63);

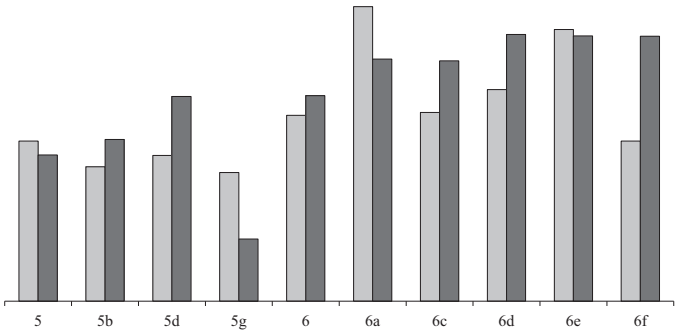


Fig. 6. Comparison between the electrochemically measured quinone reactivity toward oxygen (light gray) and ROS release in HL-60 cells (dark gray).

ROS with $-E_{plc}$ (−0.67) ($p < 0.05$) suggested that there is an inverse relationship between these variables. A statistically significant positive correlation ($p < 0.05$) was observed between the variable IC_{50} with $-E_{plc}$ (0.62) and ROS with k_{app} (0.62). These results mean that ROS is important for the cytotoxic activity. The lower the IC_{50}, the higher the ROS release. Thermodynamic and kinetic parameters also correlate; easier reduced quinones (the *ortho* ones) transfer one electron to the oxygen with a faster rate and this trend reflects in the biological activity.

The underlying rationale for this fact may be the structure of the reduced quinone. The redox-cycling of quinones *in vivo* may be initiated by either one- or two-electron reduction. The one-electron reduction of quinones is catalyzed by NADPH–cytochrome P450 reductase, generating unstable semiquinones. They transfer electrons to molecular oxygen and return to their original quinoidal formation, thus generating superoxide anion radical ($O_2^{\bullet -}$). Superoxide can dismutate into hydrogen peroxide (H_2O_2) by a SOD-catalyzed reaction, and then, by the Fenton reaction, hydroxyl radical ($HO^{\bullet}$) would be formed by the iron-catalyzed reduction of peroxide [13,34].

The electrochemical experiment mimics the one electron reduction. These results are one more evidence for the possibility of using electrochemistry for an initial screening of biological activity, as suggested before [13,14,18].

4. Conclusions

The β-dihydrofuran naphthoquinones were shown to be highly cytotoxic, while their α-isomers were shown to be less active. There is correlation among ROS release in cells, indirect electrochemical ROS release and cytotoxic activity, being higher for *ortho*-quinones, easer to reduce than the corresponding *para*-isomers.

Electrochemical methods (analytical and preparative) and electrochemical (thermodynamic and kinetic) parameters are shown to be useful in biomedical chemistry, considering the mechanisms of biological electron-transfer processes and those could be used in the planning of lead bioactive compounds, wherein the mechanism of molecular action is based on redox reactions leading to oxidative stress.

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References

[1] http://www.who.int/mediacentre/factsheets/fs297/en/index.html
[2] S.B. Ferreira, K. Salomão, F.C. Silva, A.V. Pinto, C.R. Kaiser, A.C. Pinto, V.F. Ferreira, S.L. Castro, Synthesis and anti-*Trypanosoma cruzi* activity of β-lapachone analogues, European Journal of Medical Chemistry 46 (2011) 3071.
[3] S.B. Ferreira, F.C. Silva, F.A.F.M. Bezerra, M.C.S. Lourenço, C.R. Kaiser, A.C. Pinto, V.F. Ferreira, Synthesis of alfa- and beta-pyran naphthoquinones as a new class of antitubercular agents, Archiv der Pharmazie (Weinheim) 343 (2010) 81.
[4] C.P.V. Freire, S.B. Ferreira, N.S.M. de Oliveira, A.B.J. Matsuura, I.L. Gama, F.C. da Silva, M.C.B.V. de Souza, E.S. Lima, V.F. Ferreira, Synthesis and biological evaluation of substituted α- and β-2,3-dihydrofuran naphthoquinones as potent anticandidal agentes, Medicinal Chemistry Communications 229 (1) (2010) 229.
[5] E.N. da Silva Júnior, M.C.B.V. Souza, M.C. Fernandes, R.F.S. Menna-Barreto, M.C.F.R. Pinto, F.A. Lopes, C.A. de Simone, C.K.Z. Andrade, A.V. Pinto, V.F. Ferreira, S.L. Castro, Synthesis and anti-*Trypanosoma cruzi* activity of derivatives from nor-lapachones and lapachones, Bioorganic and Medicinal Chemistry 16 (2008) 5030.
[6] M.O.F. Goulart, L.R. Freitas, J. Tonholo, F.C. de Abreu, D.S. Raslan, S. Starling, C.L. Zani, A.B. Oliveira, E. Chiari, Trypanocidal activity and redox potentials of heterocyclic and 2-hydroxy-naphthoquinones, Bioorganic and Medicinal Chemistry 7 (1997) 2043.
[7] E.N. da Silva Júnior, C.F. de Deus, B.C. Cavalcanti, C. Pessoa, L.V. Costa-Lotufo, R.C. Montenegro, M.O. Moraes, M.C.F.R. Pinto, C.A. de Simone, V.F. Ferreira, M.O.F. Goulart, C.K.Z. Andrade, A.V. Pinto, 3-Arylamino and 3-alkoxy-nor-β-lapachone derivatives: synthesis and cytotoxicity against cancer cell lines, Journal of Medicinal Chemistry 53 (2010) 504.
[8] E.N. da Silva Júnior, M.C.B.V. de Souza, A.V. Pinto, M.C.F.R. Pinto, M.O.F. Goulart, F.W.A. Barros, C. Pessoa, L.V. Costa-Lotufo, R.C. Montenegro, M.O. Moraes, V.F. Ferreira, Synthesis potent antitumor activity of new arylamino derivatives of nor-β-lapachone and nor-α-lapachone, Bioorganic and Medicinal Chemistry 15 (2007) 7035.
[9] E.N. da Silva Júnior, M.A.B.F. Moura, A.V. Pinto, M.C.F.R. Pinto, M.C.B.V. de Souza, A.J. Araújo, C. Pessoa, L.V. Costa-Lotufo, R.C. Montenegro, M.O. Moraes, V.F. Ferreira, M.O.F. Goulart, Cytotoxic trypanocidal activities physicochemical parameters of nor-β-lapachone-based 1,2,3-triazoles, Journal of the Brazilian Chemical Society 20 (2009) 635.
[10] C. Asche, Antitumour quinones, Mini-Reviews in Medicinal Chemistry 5 (2005) 449.
[11] A. Goldin, J.M. Venditti, J.S. MacDonald, F.M. Muggian, J.E. Henry, V.T. Devita, Current results of the screening program at the division of cancer treatment, National Cancer Institute, European Journal of Cancer 17 (1981) 129.
[12] D.C.M. Ferreira, I. Tapsoba, S. Arbault, Y. Bouret, A.M.S. Moreira, A.V. Pinto, M.O.F. Goulart, C. Amatore, Ex vivo activities of β-lapachone and α-lapachone on macrophages: a quantitative pharmacological analysis based on amperometric monitoring of oxidative bursts by single cells, ChemBioChem 10 (2009) 528.
[13] E.A. Hillard, F.C. de Abreu, D.C.M. Ferreira, G. Jaouen, M.O.F. Goulart, C. Amatore, Electrochemical parameters and techniques in drug development, with an emphasis on quinones and related compounds, Chemical Communications (2008) 2612.
[14] F.C. de Abreu, P.A.M. Ferraz, M.O.F. Goulart, Some applications of electrochemistry in biomedical chemistry. Emphasis on the correlation of electrochemical and bioactive properties, Journal of the Brazilian Chemical Society 13 (2002) 19.
[15] A.J. Araújo, A.A. de Souza, E.N. da Silva Júnior, J.D.B. Marinho-Filho, M.A.B.F. de Moura, D.D. Rocha, M.C. Vasconcellos, C.O. Costa, C. Pessoa, M.O. de Moraes, V.F. Ferreira, F.C. de Abreu, A.V. Pinto, R.C. Montenegro, L.V. Costa-Lotufo, M.O.F. Goulart, Growth inhibitory effects of 3′-nitro-3-phenylamino nor-beta-lapachone against HL-60: a redox-dependent mechanism, Toxicology In Vitro 26 (2012) 585.
[16] B. Cavalcanti, F.W.A. Barros, I. Cabral, J. Ferreira, H. Magalhães, H. Júnior, E.N. da Silva Jr., F.C. de Abreu, C.O. Costa, M.O.F. Goulart, M.O. Moraes, C. Pessoa, Preclinical genotoxicology of nor-β-lapachone in human cultured lymphocytes and Chinese hamster lung fibroblasts, Chemical Research in Toxicology 24 (2011) 1560.
[17] B. Halliwell, Oxidative stress and cancer: have we moved forward? Biochemical Journal 401 (2007) 1.
[18] P. Kovacic, Unifying mechanism for anticancer agents involving electron transfer and oxidative stress: clinical implications, Medical Hypotheses 69 (2007) 510.
[19] E.O. Hileman, J. Liu, M. Albitar, M.J. Keateng, P. Huang, Intrinsic oxidative stress in cancer cells: a biochemical basis for therapeutic selectivity, Cancer Chemotherapy and Pharmacology 53 (2004) 209.
[20] Y. Song, G.R. Buettner, Thermodynamic and kinetic considerations for the reaction of semiquinone radicals to form superoxide and hydrogen peroxide, Free Radical Biology and Medicine 49 (2010) 919.
[21] D.T. Sawyer, J.S. Velentine, How super is superoxide? Accounts of Chemical Research 14 (1981) 393.
[22] D. Vasudevan, H. Wendt, Electroreduction of oxygen in aprotic media, Journal of Electroanalytical Chemistry 192 (1995) 69.
[23] A. René, M.-L. Abasq, D. Hauchard, P. Hapiot, How do phenolic compounds react toward superoxide ion? A simple electrochemical method for evaluating antioxidant capacity, Analytical Chemistry 82 (20) (2010) 8703.
[24] M.O.F. Goulart, N.M.F. Lima, A.E.G. Santana, P.A.L. Ferraz, J.C.M. Cavalcanti, A. Liwo, P. Falkowsky, T. Ossowsky, Electrochemical studies of isolapachol with emphasis on oxygen interaction with its radical anions, Journal of Electroanalytical Chemistry 566 (2004) 25.
[25] M.O.F. Goulart, T. Ossowski, P. Pipka, A. Liwo, Electrochemical study of oxygen interaction with lapachol and its radical anions, Bioelectrochemistry 59 (2003) 85.

[26] T. Ossowski, P. Pipka, A. Liwo, D. Jeziorek, Electrochemical UV-spectrophotometric study of oxygen and superoxide anion radical interaction with anthraquinone derivatives and their radical anions, Electrochimica Acta 45 (2000) 3581.
[27] V. Nair, P.M. Treesa, D. Maliakal, N. Rath, CAN mediated oxidative addition of 2-hydroxynaphtoquinone to dienes: a facilite synthesis of naphthofurandiones, Tetrahedron 57 (2001) 7705.
[28] P.C. Jimenez, S.C. Fortier, T.M.C. Lotufo, C. Pessoa, M.E.A. Moraes, M.O. de Moraes, L.V. Costa-Lotufo, Biological activity in extract of Ascidians (Tunicata Ascidiacea) from northeastern Brazilian coast, Journal of Experimental Marine Biology and Ecology 287 (1) (2003) 93.
[29] T. Mosmann, Rapid colorimetric assay for cellular growth and survivor: application to proliferation and cytotoxicity assays, Journal of Immunological Methods 65 (1983) 55.
[30] C.P. LeBel, H. Ischiropoulos, S.C. Bondy, Evaluation of the probe 2′,7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress, Chemical Research in Toxicology 5 (2) (1992) 227.
[31] I.H. Hall, N.J. Peaty, J.R. Henry, J. Easmon, G. Heinisch, G. Purstinger, Investigations on the mechanism of action of the novel antitumor agents 2-benzothiazolyl, 2-benzoxazolyl, and 2-benzimidazolyl hidrazones derived from 2-acetylpyridine, Archiv der Pharmazie: Pharmaceutical and Medicinal Chemistry 332 (1999) 115.
[32] T. Ozben, Oxidative stress apoptosis impact on cancer therapy, Journal of Pharmaceutical Sciences 96 (9) (2007) 2181.
[33] J. Tonholo, L.R. Freitas, F.C. De Abreu, D.C. Azevedo, C.L. Zani, A.B. Oliveira, M.O.F. Goulart, Electrochemical properties of biologically active heterocyclic naphthoquinones, Journal of the Brazilian Chemical Society 9 (2) (1998) 163.
[34] A. Reichstein, S. Vortherms, S. Bannwitz, J. Tentrop, H. Prinz, K. Müller, Synthesis and structure–activity relationships of lapacho analogues. 1. Suppression of human keratinocyte hyperproliferation by 2-substituted naphtho[2,3-b]furan-4,9-diones, activation by enzymatic one- and two-electron reduction, and intracellular generation of superoxide, Journal of Medicinal Chemistry 55 (16) (2012) 7273.
[35] A.J. Bard, R.L. Faulkner, Electrochemical Methods, John Wiley and Sons, New York, 1980.